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Chimeric blend A

Chimeric blend B

pH 5.0 7.0 7.5 8.0 8.5 9.0 9.5 10.0 5.0 7.0 7.5 8.0 8.5 9.0 9.5 10.0 M

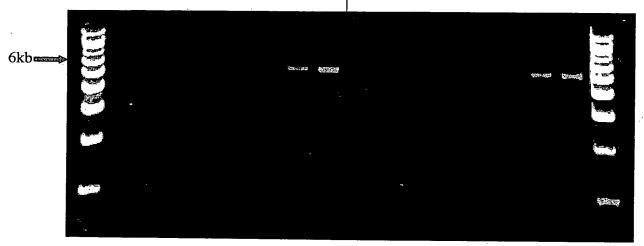


Fig. 2

6kb BG 15"/kb - 1'-30"

1X Cloned buffer

 $pH \hspace{0.5cm} 9.5 \hspace{0.5cm} 10.0 \hspace{0.5cm} 10.5 \hspace{0.5cm} 10.8 \hspace{0.5cm} 11.0 \hspace{0.5cm} 11.5 \hspace{0.5cm} 11.8 \hspace{0.5cm} 12.0$

6kb

Fig. 3

19kb BG 30"/kb - 9.5' extension

	pH 1	0.0	pH	11.8	1.5X	Cl	
M	1	2	3	4	5	6	
					Ф Ф.	the new terms of the ne	

Fig. 4

19kb BG
30"/kb - 9.5' extension

	Pfu-Sso ²	7d blend [10.0	l l	Sso7d 10.0	Herci	ulase	1	culase OMSO
M	1	2	3	. *	5	6	7	8

19kb 🛭

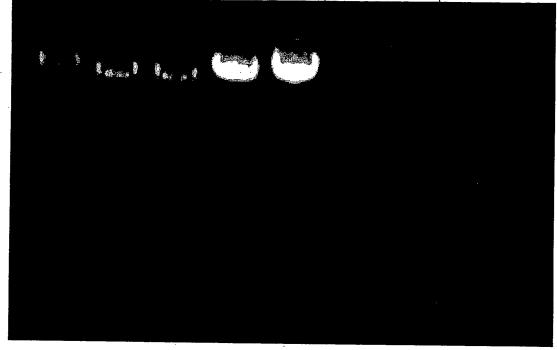


Fig. 5

19kb BG
30"/kb - 9.5' extension

		P	fu-Sso7d	l pH 10	.0	Herci		KC	DD HS	
	M	1	2	3	4	5	6	7	8	
19kb		,			Maryla Straigh					

5. 6 900bp HαΑΤ 1"/kb - 1" extension

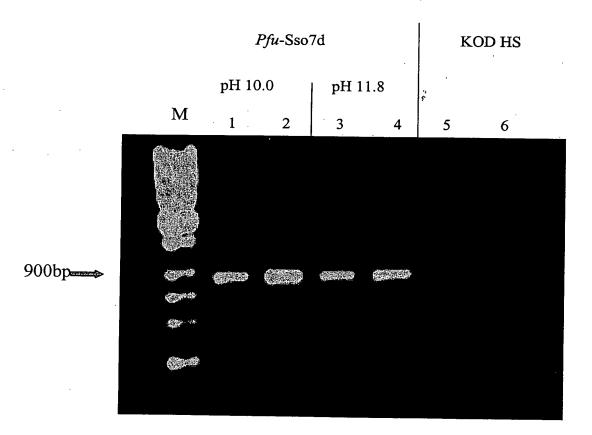


Fig.

2.6kb HαAT 2"/kb - 5" extension

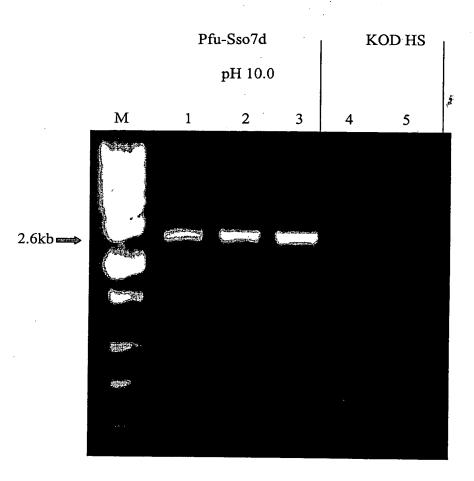


Fig. 8

6kb BG 10"/kb - 1' extension

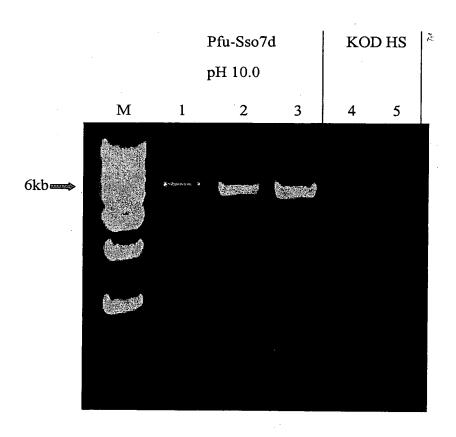


Fig. 9

2.6kb HαAT 30"/kb - 1'. 18" extension

		· .	Pfu-S pH		KO	D HS	
	M	1	2	3	4	5	
2.6kb							

Figure 10. Oligonucleotide Primers for QuikChange Mutagenesis

V93E#1

5'-gAACATCCCCAAgATgAACCCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 6)

V93E#2

5'-CTTTTTCTCTAATAgTgggTTCATCTTgggggATgTTC-3' (SEQ ID NO: 7)

V93R#1

5'-gAACATCCCCAAgATAgACCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 8)

V93R#2

5'-CTTTTTCTCTAATAgTgggTCTATCTTgggggATgTTC-3' (SEQ ID NO: 9)

V93N#1

5'-gAACATCCCCAAgATAACCCCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 10)

V93N#2

5'-CTTTTTCTCTAATAgTggggTTATCTTgggggATgTTC-3' (SEQ ID NO: 11)

V93H#1

5'-gAACATCCCCAAgAT<u>CAC</u>CCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 12)

V93H#2

5'-CTTTTTCTCTAATAgTggggTgATCTTgggggATgTTC-3' (SEQ ID NO: 13)

V93X (for saturation mutagenesis; obtained V93G and V93L mutants from library)

5'-(Phosphate)gAACATCCCCAAgAT<u>NNK</u>CCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 14)

V93K#1

Fig. 10 (wit)

5'-gAACATCCCCAAgATAAACCCACTATTAgAg-3' (SEQ ID NO: 15)

V93K#2

5'-CTCTAATAgTgggTTTATCTTgggggATgTTC-3' (SEQ ID NO: 16)

QCM#1

 $\verb|5'-(Phosphate)| gAACATCCCCAAgATgCACCCACTATTAgAgAAAAAg-(SEQ~ID~N)|$

17)'

Alanine

QCM#2

5'-(Phosphate)gAACATCCCCAAgATgACCCCACTATTAgAgAAAAAg-3'(SEQ ID

NO: 18)

Aspartic Acid

QCM#3

5'-(Phosphate)gAACATCCCCAAgATTgCCCCCACTATTAgAgAAAAAg-3' (SEO I

NO: 19)

Cysteine

QCM#4

5'-

(Phosphate)gAACATCCCCAAgATATACCCACTATTAgAgAAAAAg-3' (SEQ ID NO: 20)

Isoleucine

QCM#5

5'-(Phosphate)gAACATCCCCAAgATATgCCCACTATTAgAgAAAAAg-3' (SEQ ID

NO: 21)

Methionine

QCM#6

5'-(Phosphate)gAACATCCCCAAgATTTCCCCACTATTAgAgAAAAAg-3' (SEQ ID

NO: 22)

Phenylalanine

Fig 10 (cont)

QCM#7

5'-(Phosphate)gAACATCCCCAAgATCCTCCCACTATTAgAgAAAAAg-3' (SEQ ID

NO: 23)

Proline

QCM#8

5'-(Phosphate)gAACATCCCCAAgATAgCCCCACTATTAgAgAAAAAg-3'

(SEQ ID NO: 23)

Serine

QCM#9

5'-(Phosphate)gAACATCCCCAAgATACACCCACTATTAgAgAAAAAg- 3'

*

(SEQ ID NO: 24)

Threonine

QCM#10

5'-(Phosphate)gAACATCCCCAAgATTACCCCACTATTAgAgAAAAAg-3'

(SEQ ID NO: 25)

Tyrosine

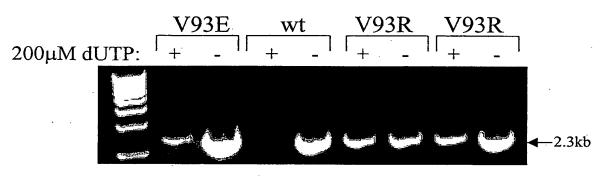
QCM#11

5'-(Phosphate)gAACATCCCCAAgATTggCCCACTATTAgAgAAAAAg-3'

(SEQ ID NO: 26)

Tryptophan

a.)



b.)

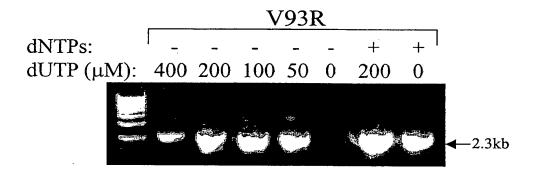


Figure 11

Pfu V93R Pfu PfuTurbo

0.3 0.5 1 2 4 0.3 0.5 1 2 4 0.3 0.5 1 2 4 U

Figure 12

FIGURE 13A

PFU DNA POLYMERASE V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC 360 CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA GAGTTTGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC 540 GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG 720 ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900 AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCCTAG 2328

PFU DNA POLYMERASE

G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

ATGATTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGÀ AACTTTATTT GGAACATCCC CAAGATXXXC CCACTATTAG AGAAAAAGTT 300

Fig. 13A (cont)

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AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC 360
CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420
GATATAGAAA CCCTCTATCA CGAAGGAGAA GAGTTTGGAA AAGGCCCAAT TATAATGATT 480
AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC 540
GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600
AAGGATCCTG ACATTATAGT TACTTATAAT GGAGACTCAT TCGCATTCCC ATATTTAGCG 660
AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG 720
ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780
TATCATGTAA TAACAAGGAC AATAAATCTC CCAACATACA CACTAGAGGC TGTATATGAA 840
GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900
AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960
GAACTCGGGA AAGAATTCCT TCCAATGGAA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020
TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA 1080
GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140
CTCAGGGAGA GCTACACAC NGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200
ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA CAATGTTTCT 1260
CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320
AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380
AGACAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CTATAGAAAA AATACTCCTT 1440
GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500
GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560
TACATCGAGT TAGTATGGAA GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620
GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 1680
GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740
GAAGGGTTTT ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800
GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TGAAATTGCA 1860
AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920
GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT; ATGAAATTCC ACCAGAGAAG 1980
CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040
GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100
GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160
TACGATCCCA AAAAGCACAA GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220
GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG 2280
ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCCTAG 2328
```

PFU DNA POLYMERASE

D141A/E143A Mutant (GCN is the codon for alanine where N=C, G, A, or T)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

ATGATTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60
AAAGAGAACG GAAAATTTAA GATAGAGCAT GATAGAACTT TTAGACCATA CATTTACGCT 120
CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180
AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240
ACCGTGTGGA AACTTTATTT GGAACATCCC CAAGATXXXC CCACTATTAG AGAAAAAGTT 300
AGAGAACATC CAGCAGTTGT GGACATCTC GAATACGATA TTCCATTTGC AAAGAGATAC 360
CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420
GCNATAGCNA CCCTCTATCA CGAAGGAGAA GAGTTTGGAA AAGGCCCAAT TATAATGATT 480
AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC 540
GTTGAGGTTG TATCAAGCGA GAGAGAGAGA ATAAAAGAGAT TCTCCAGGAT TATCAGGGAG 600
AAGGATCCTG ACATTATAGT TACTTATAAT GGAGACTCAT TCGCATTCCC ATATTTAGCG 660
AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG 720
ATGCAGAGAA TAGCGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780
TATCATGTAA TAACAAGGAC AATAAATCTC CCAACATACA CACTAGAGGC TGTATATGAA 840
GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAA AGCCTGGGAA 900

Fig 13A (cont)

AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAAT AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCCTAG 2328

KOD DNA POLYMERASE

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC CTGTCATAAG AATTTTCAAG 60 AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT TTGAACCCTA CTTCTACGCC 120 CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA TAACCGCCGA GAGGCACGGG 180 ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA AGTTCCTCGG GAGACCAGTT 240 GAGGTCTGGA AACTCTACTT TACTCATCCG CAGGACXXXC CAGCGATAAG GGACAAGATA 300 CGAGAGCATC CAGCAGTTAT TGACATCTAC GAGTACGACA TACCCTTCGC CAAGCGCTAC 360 CTCATAGACA AGGGATTAGT GCCAATGGAA GGCGACGAGG AGCTGAAAAT GCTCGCCTTC 420 GACATTGAAA CTCTCTACCA TGAGGGCGAG GAGTTCGCCG AGGGGCCAAT CCTTATGATA 480 AGCTACGCCG ACGAGGAAGG GGCCAGGGTG ATAACTTGGA AGAACGTGGA TCTCCCCTAC 540 GTTGACGTCG TCTCGACGGA GAGGGAGATG ATAAAGCGCT TCCTCCGTGT TGTGAAGGAG 600 AAAGACCCGG ACGTTCTCAT AACCTACAAC GGCGACAACT TCGACTTCGC CTATCTGAAA 660 AAGCGCTGTG AAAAGCTCGG AATAAACTTC GCCCTCGGAA GGGATGGAAG CGAGCCGAAG 720 ATTCAGAGGA TGGGCGACAG GTTTGCCGTC GAAGTGAAGG GACGGATACA CTTCGATCTC 780 TATCCTGTGA TAAGACGGAC GATAAACCTG CCCACATACA CGCTTGAGGC CGTTTATGAA 840 GCCGTCTTCG GTCAGCCGAA GGAGAAGGTT TACGCTGAGG AAATAACCAC AGCCTGGGAA 900 ACCGGCGAGA ACCTTGAGAG AGTCGCCCGC TACTCGATGG AAGATGCGAA GGTCACATAC 960 GAGCTTGGGA AGGAGTTCCT TCCGATGGAG GCCCAGCTTT CTCGCTTAAT CGGCCAGTCC 1020 CTCTGGGACG TCTCCCGCTC CAGCACTGGC AACCTCGTTG AGTGGTTCCT CCTCAGGAAG 1080 GCCTATGAGA GGAATGAGCT GGCCCCGAAC ÁAGCCCGATG AAAAGGAGCT GGCCAGAAGA 1140 CGGCAGAGCT ATGAAGGAGG CTATGTAAAA GAGCCCGAGA GAGGGTTGTG GGAGAACATA 1200 GTGTACCTAG ATTTTAGATC CCTGTACCCC TCAATCATCA TCACCCACAA CGTCTCGCCG 1260 GATACGCTCA ACAGAGAAGG ATGCAAGGAA TATGACGTTG CCCCACAGGT CGGCCACCGC 1320 CAGAAGATAA AGAAGAAGAT GAAGGCCACG ATTGACCCGA TCGAGAGGAA GCTCCTCGAT 1440 TACAGGCAGA GGGCCATCAA GATCCTGGCA AACAGCTACT ACGGTTACTA CGGCTATGCA 1500 AGGGCGCGCT GGTACTGCAA GGAGTGTGCA GAGAGCGTAA CGGCCTGGGG AAGGGAGTAC 1560 ATAACGATGA CCATCAAGGA GATAGAGGAA AAGTACGGCT TTAAGGTAAT CTACAGCGAC 1620

Fig 13A (cont)

ACCGACGGAT TTTTTGCCAC AATACCTGGA GCCGATGCTG AAACCGTCAA AAAGAAGGCT 1680
ATGGAGTTCC TCAAGTATAT CAACGCCAAA CTTCCGGGCG CGCTTGAGCT CGAGTACGAG 1740
GGCTTCTACA AACGCGGCTT CTTCGTCACG AAGAAGAAGT ATGCGGTGAT AGACGAGGAA 1800
GGCAAGATAA CAACGCGCGG ACTTGAGATT GTGAGGCGTG ACTGGAGCGA GATAGCGAAA 1860
GAGACGCAGG CGAGGGTTCT TGAAGCTTTG CTAAAGGACG GTGACGTCGA GAAGGCCGTG 1920
AGGATAGTCA AAGAAGTTAC CGAAAAGCTG AGCAAGTACG AGGTTCCGCC GGAGAAGCTG 1980
GTGATCCACG AGCAGATAAC GAGGGATTTA AAGGACTACA AGGCAACCGG TCCCCACGTT 2040
GCCGTTGCCA AGAGGTTGGC CGCGAGAGGA GTCAAAATAC GCCCTGGAAC GGTGATAAGC 2100
TACATCGTGC TCAAGGGCTC TGGGAGGATA GGCGACAGGG CGATACCGTT CGACGAGTTC 2160
GACCCGACGA AGCACAAGTA CGACGCCGAG TACTACATTG AGAACCAGGT TCTCCCAGCC 2220
GTTGAGAGAA TTCTGAGAGC CTTCGGTTAC CGCAAGGGAA CCTTGA 2325

Vent DNA POLYMERASE

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CTATAATCCG AATTTTTAAG 60 AAAGAGAACG GGGAGTTTAA AATAGAACTT GACCCTCATT TTCAGCCCTA TATATATGCT 120 CTTCTCAAAG ATGACTCCGC TATTGAGGAG ATAAAGGCAA TAAAGGGCGA GAGACATGGA 180 AAAACTGTGA GAGTGCTCGA TGCAGTGAAA GTCAGGAAAA AATTTTTGGG AAGGGAAGTT 240 GAAGTCTGGA AGCTCATTTT CGAGCATCCC CAAGACXXXC CAGCTATGCG GGGCAAAATA 300 AGGGAACATC CAGCTGTGGT TGACATTTAC GAATATGACA TACCCTTTGC CAAGCGTTAT 360 CTCATAGACA AGGGCTTGAT TCCCATGGAG GGAGACGAGG AGCTTAAGCT CCTTGCCTTT 420 GATATTGAAA CGTTTTATCA TGAGGGAGAT GAATTTGGAA AGGGCGAGAT AATAATGATT 480 AGTTATGCCG ATGAAGAAGA GGCCAGAGTA ATCACATGGA AAAATATCGA TTTGCCGTAT 540 GTCGATGTTG TGTCCAATGA AAGAGAAATG ATAAAGCGTT TTGTTCAAGT TGTTAAAGAA 600 AAAGACCCCG ATGTGATAAT AACTTACAAT GGGGACAATT TTGATTTGCC GTATCTCATA 660 AAACGGCAG AAAAGCTGGG AGTTCGGCTT GTCTTAGGAA GGGACAAAGA ACATCCCGAA 720 CCCAAGATTC AGAGGATGGG TGATAGTTTT GCTGTGGAAA TCAAGGGTAG AATCCACTTT 780 GATCTTTTCC CAGTTGTGCG AAGGACGATA AACCTCCCAA CGTATACGCT TGAGGCAGTT 840 TATGAAGCAG TTTTAGGAAA AACCAAAAGC AAATTAGGAG CAGAGGAAAT TGCCGCTATA 900 TGGGAAACAG AAGAAAGCAT GAAAAAACTA GCCCAGTACT CAATGGAAGA TGCTAGGGCA 960 ACGTATGAGC TCGGGAAGGA ATTCTTCCCC ATGGAAGCTG AGCTGGCAAA GCTGATAGGT 1020 CAAAGTGTAT GGGACGTCTC GAGATCAAGC ACCGGCAACC TCGTGGAGTG GTATCTTTTA 1080 AGGGTGGCAT ACGCGAGGAA TGAACTTGCA CCGAACAAAC CTGATGAGGA AGAGTATAAA 1140 CGGCGCTTAA GAACAACTTA CCTGGGAGGA TATGTAAAAG AGCCAGAAAA AGGTTTGTGG 1200 GAAAATATCA TTTATTTGGA TTTCCGCAGT CTGTACCCTT CAATAATAGT TACTCACAAC 1260 GTATCCCCAG ATACCCTTGA AAAAGAGGGC TGTAAGAATT ACGATGTTGC TCCGATAGTA 1320 GGATATAGGT TCTGCAAGGA CTTTCCGGGC TTTATTCCCT CCATACTCGG GGACTTAATT 1380 GCAATGAGGC AAGATATAAA GAAGAAAATG AAATCCACAA TTGACCCGAT CGAAAAGAAA 1440 ATGCTCGATT ATAGGCAAAG GGCTATTAAA TTGCTTGCAA ACAGCTATTA CGGCTATATG 1500 GGGTATCCTA AGGCAAGATG GTACTCGAAG GAATGTGCTG AAAGCGTTAC CGCATGGGGG 1560 AGACACTACA TAGAGATGAC GATAAGAGAA ATAGAGGAAA AGTTCGGCTT TAAGGTTCTT 1620 TATGCGGACA CTGACGGCTT TTATGCCACA ATACCCGGGG AAAAGCCTGA ACTCATTAAA 1680 AAGAAAGCCA AGGAATTCCT AAACTACATA AACTCCAAAC TTCCAGGTCT GCTTGAGCTT 1740 GAGTATGAGG GCTTTTACTT GAGAGGATTC TTTGTTACAA AAAAGCGCTA TGCAGTCATA 1800 GATGAAGAGG GCAGGATAAC AACAAGGGGC TTGGAAGTAG TAAGGAGAGA TTGGAGTGAG 1860 ATAGCTAAGG AGACTCAGGC AAAGGTTTTA GAGGCTATAC TTAAAGAGGG AAGTGTTGAA 1920 AAAGCTGTAG AAGTTGTTAG AGATGTTGTA GAGAAAATAG CAAAATACAG GGTTCCACTT 1980 GAAAAGCTTG TTATCCATGA GCAGATTACC AGGGATTTAA AGGACTACAA AGCCATTGGC 2040 CCTCATGTCG CGATAGCAAA AAGACTTGCC GCAAGAGGGA TAAAAGTGAA ACCGGGCACA 2100 ATAATAAGCT ATATCGTTCT CAAAGGGAGC GGAAAGATAA GCGATAGGGT AATTTTACTT 2160 ACAGAATACG ATCCTAGAAA ACACAAGTAC GATCCGGACT ACTACATAGA AAACCAAGTT 2220 TTGCCGGCAG TACTTAGGAT ACTCGAAGCG TTTGGATACA GAAAGGAGGA TTTAAGGTAT 2280 CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GGTAG 2325

Fig 13A (cont)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG 60 AAAGAAACG GCGAGTTTAA GGTTGAGTAC GACAGAAACT TTAGACCTTA CATTTACGCT 120 CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGG 180 AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA AGTTCCTGGG GAGGCCGATT 240 GAGGTATGGA GGCTGTACTT TGAACACCCT CAGGACXXXC CCGCAATAAG GGATAAGATA 300 AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGTACGACA TTCCGTTCGC GAAGAGGTAC 360 CTAATAGACA AAGGCCTAAT TCCAATGGAA GGCGATGAAG AGCTCAAGTT GCTCGCATTT 420 GACATAGAAA CCCTCTATCA CGAAGGGGAG GAGTTCGCGA AGGGGCCCAT TATAATGATA 480 AGCTATGCTG ATGAGGAAGA AGCCAAAGTC ATAACGTGGA AAAAGATCGA TCTCCCGTAC GTCGAGGTAG TTTCCAGCGA GAGGGAGATG ATAAAGCGGT TCCTCAAGGT GATAAGGGAG 600 AAAGATCCCG ATGTTATAAT TACCTACAAC GGCGATTCTT TCGACCTTCC CTATCTAGTT 660 AAGAGGCCG AAAAGCTCGG GATAAAGCTA CCCCTGGGAA GGGACGGTAG TGAGCCAAAG 720 ATGCAGAGGC TTGGGGATAT GACAGCGGTG GAGATAAAGG GAAGGATACA CTTTGACCTC 780 TACCACGTGA TTAGGAGAAC GATAAACCTC CCAACATACA CCCTCGAGGC AGTTTATGAG 840 GCAATCTTCG GAAAGCCAAA GGAGAAAGTT TACGCTCACG AGATAGCTGA GGCCTGGGAG 900 ACTGGAAAGG GACTGGAGAG AGTTGCAAAG TATTCAATGG AGGATGCAAA GGTAACGTAC 960 GAGCTCGGTA GGGAGTTCTT CCCAATGGAG GCCCAGCTTT CAAGGTTAGT CGGCCAGCCC 1020 CTGTGGGATG TTTCTAGGTC TTCAACTGGC AACTTGGTGG AGTGGTACCT CCTCAGGAAG 1080 GCCTACGAGA GGAATGAATT GGCTCCAAAC AAGCCGGATG AGAGGGAGTA CGAGAGAAGG 1140 CTAAGGGAGA GCTACGCTGG GGGATACGTT AAGGAGCCGG AGAAAGGGCT CTGGGAGGGG 1200 TTAGTTTCCC TAGATTTCAG GAGCCTGTAC CCCTCGATAA TAATCACCCA TAACGTCTCA 1260 CCGGATACGC TGAACAGGGA AGGGTGTAGG GAATACGATG TCGCCCCAGA GGTTGGGCAC AAGTTCTGCA AGGACTTCCC GGGGTTTATC CCCAGCCTGC TCAAGAGGTT ATTGGATGAA AGGCAAGAAA TAAAAAGGAA GATGAAAGCT TCTAAAGACC CAATCGAGAA GAAGATGCTT GATTACAGGC AACGGGCAAT CAAAATCCTG GCAAACAGCT ATTATGGGTA TTATGGGTAC GCAAAAGCCC GTTGGTACTG TAAGGAGTGC GCAGAGAGCG TTACGGCCTG GGGGAGGGAA TATATAGAGT TCGTAAGGAA GGAACTGGAG GAAAAGTTCG GGTTCAAAGT CTTATACATA GACACAGATG GACTCTACGC CACAATTCCT GGGGCAAAAC CCGAGGAGAT AAAGAAGAAA GCCCTAGAGT TCGTAGATTA TATAAACGCC AAGCTCCCAG GGCTGTTGGA GCTTGAGTAC GAGGGCTTCT ACGTGAGAGG GTTCTTCGTG ACGAAGAAGA AGTATGCGTT GATAGATGAG GAAGGGAAGA TAATCACTAG GGGGCTTGAA ATAGTCAGGA GGGACTGGAG CGAAATAGCC AAAGAAACCC AAGCAAAAGT CCTAGAGGCT ATCCTAAAGC ATGGCAACGT TGAGGAGGCA GTAAAGATAG TTAAGGAGGT AACTGAAAAG CTGAGCAAGT ACGAAATACC TCCAGAAAAG CTAGTTATTT ACGAGCAGAT CACGAGGCCC CTTCACGAGT ACAAGGCTAT AGGTCCGCAC GTTGCCGTGG CAAAAAGGTT AGCCGCTAGA GGAGTAAAGG TGAGGCCTGG CATGGTGATA GGGTACATAG TGCTGAGGGG AGACGGGCCA ATAAGCAAGA GGGCTATCCT TGCAGAGGAG TTCGATCTCA GGAAGCATAA GTATGACGCT GAGTATTACA TAGAAAATCA GGTTTTACCT GCCGTTCTTA GAATATTAGA GGCCTTTGGG TACAGGAAAG AAGACCTCAG GTGGCAGAAG 2280

JDF-3

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

ACTAAACAGA CAGGTCTTAC GGCATGGCTT AACATCAAGA AGAAGTAA

2328

Fig 13A (unt)

GAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCA TAAGGCGCACCATAAACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCAAGGAGAAGGT CTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGCTTGAGAGGGTCGCGCGCTACTCGATGGAGGACGCG AGGGTTACCTACGAGCTTGGCAGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGG CAACAAGCCCGACGAGAGGGGGCTGGCGAGGAGAAGGGGGGGCTACGCCGGTGGCTACGTCAAGGAGCCGGAGCGGGGA CTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCTTCAATCATCACCCCACAACGTCTCGCCAGATA GGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGAGTACATCGAAATGGTCATCAGAGA GCTTGAGGAAAAGTTCGGTTTTAAAGTCCTCTATGCAGACACAGAĈGGTCTCCATGCCACCATTCCTGGAGCGGACGCT GAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTCGAACTCGAATACG AGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCAAGATAACCACGCG CGGGCTTGAGATAGTCAGGCGCGACTGGAGCGAGATAGCGAAGGAGACGCAGGCGAGGGTTTTGGAGGCGATACTCAGG CACGGTGACGTTGAAGAGGCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAAGTACGAGGTTCCGCCGGAGA AGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGCCCCCACGTAGCCATAGCGAAqcG TTTGGCCGCCAGAGGTGTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGC GACAGGGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATGCGGACTACTACATCGAGAACCAGGTTC TGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGTCGG GCTTGGCGCGTGGCTGAAGCCGAAGGGGAAGAAGAAGTGA

Figure 13B

>Pfu V93R

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDRPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFDIETLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TGGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYËQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS //

>Pfu V93E

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDEPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFDIETLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TGGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS

>Pfu V93R/G387P

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDRPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFDIETLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TPGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS

>Pfu V93R/D141A/E143A

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDRPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFAIATLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TGGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS

>Pfu V93E/G387P

Fig. 13B (cont)

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDEPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFDIETLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TPGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS

>Pfu V93E/D141A/E143A

£

MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYIYALLRDDSKIEEVKKITGERHGKIVRIVDVEKVEKKFLG
KPITVWKLYLEHPQDRPTIREKVREHPAVVDIFEYDIPFAKRYLIDKGLIPMEGEEELKILAFAIATLYHEGEEFGK
GPIIMISYADENEAKVITWKNIDLPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLT
IGRDGSEPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEIAKAWESGENLERV
AKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGNLVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESY
TGGFVKEPEKGLWENIVYLDFRALYPSIIITHNVSPDTLNLEGCKNYDIAPQVGHKFCKDIPGFIPSLLGHLLEERQ
KIKTKMKETQDPIEKILLDYRQKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIELVWKELEEKFGFKVLY
IDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYEGFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDW
SEIAKETQARVLETILKHGDVEEAVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVK
IKPGMVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKEDLRYQKTRQVGLTSWL
NIKKS

>DEEP VENT V93R

MILDADYITEDGKPIIRIFKKENGEFKVEYDRNFRPYIYALLKDDSQIDEVRKITAERHGKIVRIIDAEKVRKKFLG
RPIEVWRLYFEHPQDRPAIRDKIREHSAVIDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFDIETLYHEGEEFAK
GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKVIREKDPDVIITYNGDSFDLPYLVKRAEKLGIKLP
LGRDGSEPKMQRLGDMTAVEIKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYAHEIAEAWETGKGLERV
AKYSMEDAKVTYELGREFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWYLLRKAYERNELAPNKPDEREYERRLRESY
AGGYVKEPEKGLWEGLVSLDFRSLYPSIIITHNVSPDTLNREGCREYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ
EIKRKMKASKDPIEKKMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVRKELEEKFGFKVLY
IDTDGLYATIPGAKPEEIKKKALEFVDYINAKLPGLLELEYEGFYVRGFFVTKKKYALIDEEGKIITRGLEIVRRDW
SEIAKETQAKVLEAILKHGNVEEAVKIVKEVTEKLSKYEIPPEKLVIYEQITRPLHEYKAIGPHVAVAKRLAARGVK
VRPGMVIGYIVLRGDGPISKRAILAEEFDLRKHKYDAEYYIENQVLPAVLRILEAFGYRKEDLRWQKTKQTGLTAWL
NIKKK

>DEEP VENT V93E

MILDADYITEDGKPIIRIFKKENGEFKVEYDRNFRPYIYALLKDDSQIDEVRKITAERHGKIVRIIDAEKVRKKFLG RPIEVWRLYFEHPQDEPAIRDKIREHSAVIDIFEYDIPFAKRYLIDKGLIPMEGDEELKLLAFDIETLYHEGEEFAK GPIIMISYADEEEAKVITWKKIDLPYVEVVSSEREMIKRFLKVIREKDPDVIITYNGDSFDLPYLVKRAEKLGIKLP LGRDGSEPKMQRLGDMTAVEIKGRIHFDLYHVIRRTINLPTYTLEAVYEAIFGKPKEKVYAHEIAEAWETGKGLERV AKYSMEDAKVTYELGREFFPMEAQLSRLVGQPLWDVSRSSTGNLVEWYLLRKAYERNELAPNKPDEREYERRLRESY AGGYVKEPEKGLWEGLVSLDFRSLYPSIIITHNVSPDTLNREGCREYDVAPEVGHKFCKDFPGFIPSLLKRLLDERQ EIKRKMKASKDPIEKKMLDYRQRAIKILANSYYGYYGYAKARWYCKECAESVTAWGREYIEFVRKELEEKFGFKVLY IDTDGLYATIPGAKPEEIKKKALEFVDYINAKLPGLLELEYEGFYVRGFFVTKKKYALIDEEGKIITRGLEIVRDW SEIAKETQAKVLEAILKHGNVEEAVKIVKEVTEKLSKYEIPPEKLVIYEQITRPLHEYKAIGPHVAVAKRLAARGVK VRPGMVIGYIVLRGDGPISKRAILAEEFDLRKHKYDAEYYIENQVLPAVLRILEAFGYRKEDLRWQKTKQTGLTAWL NIKKK

>TGO V93R

MILDTDYITEDGKPVIRIFKKENGEFKIDYDRNFEPYIYALLKDDSAIEDVKKITAERHGTTVRVVRAEKVKKKFLG RPIEVWKLYFTHPQDRPAIRDKIKEHPAVVDIYEYDIPFAKRYLIDKGLIPMEGDEELKMLAFDIETLYHEGEEFAE GPILMISYADEEGARVITWKNIDLPYVDVVSTEKEMIKRFLKVVKEKDPDVLITYNGDNFDFAYLKKRSEKLGVKFI

Fig 13B (cont)

LGREGSEPKIQRMGDRFAVEVKGRIHFDLYPVIRRTINLPTYTLEAVYEAIFGQPKEKVYAEEIAQAWETGEGLERV ARYSMEDAKVTYELGKEFFPMEAQLSRLVGQSLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDERELARRRESYA GGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNREGCEEYDVAPQVGHKFCKDFPGFIPSLLGDLLEERQK VKKKMKATIDPIEKKLLDYRQRAIKILANSFYGYYGYAKARWYCKECAESVTAWGRQYIETTIREIEEKFGFKVLYA DTDGFFATIPGADAETVKKKAKEFLDYINAKLPGLLELEYEGFYKRGFFVTKKKYAVIDEEDKITTRGLEIVRRDWS EIAKETQARVLEAILKHGDVEEAVRIVKEVTEKLSKYEVPPEKLVIYEQITRDLKDYKATGPHVAVAKRLAARGIKI RPGTVISYIVLKGSGRIGDRAIPFDEFDPAKHKYDAEYYIENQVLPAVERILRAFGYRKEDLRYQKTRQVGLGAWLK PKT

>TGO V93E

MILDTDYITEDGKPVIRIFKKENGEFKIDYDRNFEPYIYALLKDDSAIEDVKKITAERHGTTVRVVRAEKVKKKFLG
RPIEVWKLYFTHPQDEPAIRDKIKEHPAVVDIYEYDIPFAKRYLIDKGLIPMEGDEELKMLAFDIETLYHEGEEFAE
GPILMISYADEEGARVITWKNIDLPYVDVVSTEKEMIKRFLKVVKEKDPDVLITYNGDNFDFAYLKKRSEKLGVKFI
LGREGSEPKIQRMGDRFAVEVKGRIHFDLYPVIRRTINLPTYTLEAVYEAIFGQPKEKVYAEEIAQAWETGEGLERV
ARYSMEDAKVTYELGKEFFPMEAQLSRLVGQSLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDERELARRRESYA
GGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNREGCEEYDVAPQVGHKFCKDFPGFIPSLLGDLLEERQK
VKKKMKATIDPIEKKLLDYRQRAIKILANSFYGYYGYAKARWYCKECAESVTAWGRQYIETTIREIEEKFGFKVLYA
DTDGFFATIPGADAETVKKKAKEFLDYINAKLPGLLELEYEGFYKRGFFVTKKKYAVIDEEDKITTRGLEIVRRDWS
EIAKETQARVLEAILKHGDVEEAVRIVKEVTEKLSKYEVPPEKLVIYEQITRDLKDYKATGPHVAVAKRLAARGIKI
RPGTVISYIVLKGSGRIGDRAIPFDEFDPAKHKYDAEYYIENQVLPAVERILRAFGYRKEDLRYQKTRQVGLGAWLK

>KOD V93R

MILDTDYITEDGKPVIRIFKKENGEFKIEYDRTFEPYFYALLKDDSAIEEVKKITAERHGTVVTVKRVEKVQKKFLG
RPVEVWKLYFTHPQDRPAIRDKIREHGAVIDIYEYDIPFAKRYLIDKGLVPMEGDEELKMLAFDIQTLYHEGEEFAE
GPILMISYADEEGARVITWKNVDLPYVDVVSTEREMIKRFLRVVKEKDPDVLITYNGDNFDFAYLKKRCEKLGINFA
LGRDGSEPKIQRMGDRFAVEVKGRIHFDLYPVIRRTINLPTYTLEAVYEAVFGQPKEKVYAEEITPAWETGENLERV
ARYSMEDAKVTYELGKEFLPMEAQLSRLIGQSLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEKELARRRQSYE
GGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNREGCKEYDVAPQVGHRFCKDFPGFIPSLLGDLLEERQK
IKKKMKATIDPIERKLLDYRQRAIKILANSYYGYYGYARARWYCKECAESVTAWGREYITMTIKEIEEKYGFKVIYS
DTDGFFATIPGADAETVKKKAMEFLNYINAKLPGALELEYEGFYKRGFFVTKKKYAVIDEEGKITTRGLEIVRRDWS
EIAKETQARVLEALLKDGDVEKAVRIVKEVTEKLSKYEVPPEKLVIHEQITRDLKDYKATGPHVAVAKRLAARGVKI
RPGTVISYIVLKGSGRIGDRAIPFDEFDPTKHKYDAEYYIENQVLPAVERILRAFGYRKEDLRYQKTRQVGLSAWLK
PKGT

>KOD V93E

MILDTDYITEDGKPVIRIFKKENGEFKIEYDRTFEPYFYALLKDDSAIEEVKKITAERHGTVVTVKRVEKVQKKFLG
RPVEVWKLYFTHPQDEPAIRDKIREHGAVIDIYEYDIPFAKRYLIDKGLVPMEGDEELKMLAFDIQTLYHEGEEFAE
GPILMISYADEEGARVITWKNVDLPYVDVVSTEREMIKRFLRVVKEKDPDVLITYNGDNFDFAYLKKRCEKLGINFA
LGRDGSEPKIQRMGDRFAVEVKGRIHFDLYPVIRRTINLPTYTLEAVYEAVFGQPKEKVYAEEITPAWETGENLERV
ARYSMEDAKVTYELGKEFLPMEAQLSRLIGQSLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDEKELARRRQSYE
GGYVKEPERGLWENIVYLDFRSLYPSIIITHNVSPDTLNREGCKEYDVAPQVGHRFCKDFPGFIPSLLGDLLEERQK
IKKKMKATIDPIERKLLDYRQRAIKILANSYYGYYGYARARWYCKECAESVTAWGREYITMTIKEIEEKYGFKVIYS
DTDGFFATIPGADAETVKKKAMEFLNYINAKLPGALELEYEGFYKRGFFVTKKKYAVIDEEGKITTRGLEIVRRDWS
EIAKETQARVLEALLKDGDVEKAVRIVKEVTEKLSKYEVPPEKLVIHEQITRDLKDYKATGPHVAVAKRLAARGVKI
RPGTVISYIVLKGSGRIGDRAIPFDEFDPTKHKYDAEYYIENQVLPAVERILRAFGYRKEDLRYQKTRQVGLSAWLK
PKGT

>VENT V93R

MILDTDYITKDGKPIIRIFKKENGEFKIELDPHFQPYIYALLKDDSAIEEIKAIKGERHGKTVRVLDAVKVRKKFLG REVEVWKLIFEHPQDRPAMRGKIREHPAVVDIYEYDIPFAKRYLIDKGLIPMEGDEELKLLAFDIETFYHEGDEFGK GEIIMISYADEEEARVITWKNIDLPYVDVVSNEREMIKRFVQVVKEKDPDVIITYNGDNFDLPYLIKRAEKLGVRLV LGRDKEHPEPKIQRMGDSFAVEIKGRIHFDLFPVVRRTINLPTYTLEAVYEAVLGKTKSKLGAEEIAAIWETEESMK

Fig 13B (cont)

KLAQYSMEDARATYELGKEFFPMEAELAKLIGQSVWDVSRSSTGNLVEWYLLRVAYARNELAPNKPDEEEYKRRLRT
TYLGGYVKEPEKGLWENIIYLDFRSLYPSIIVTHNVSPDTLEKEGCKNYDVAPIVGYRFCKDFPGFIPSILGDLIAM
RQDIKKKMKSTIDPIEKKMLDYRQRAIKLLANSYYGYMGYPKARWYSKECAESVTAWGRHYIEMTIREIEEKFGFKV
LYADTDGFYATIPGEKPELIKKKAKEFLNYINSKLPGLLELEYEGFYLRGFFVTKKRYAVIDEEGRITTRGLEVVRR
DWSEIAKETQAKVLEAILKEGSVEKAVEVVRDVVEKIAKYRVPLEKLVIHEQITRDLKDYKAIGPHVAIAKRLAARG
IKVKPGTIISYIVLKGSGKISDRVILLTEYDPRKHKYDPDYYIENQVLPAVLRILEAFGYRKEDLRYQSSKQTGLDA
WLKR

>VENT V93E

MILDTDYITKDGKPIIRIFKKENGEFKIELDPHFQPYIYALLKDDSAIEEIKAIKGERHGKTVRVLDAVKVRKKFLG
REVEVWKLIFEHPQDEPAMRGKIREHPAVVDIYEYDIPFAKRYLIDKGLIPMEGDEELKLLAFDIETFYHEGDEFGK
GEIIMISYADEEEARVITWKNIDLPYVDVVSNEREMIKRFVQVVKEKDPDVIITYNGDNFDLPYLIKRAEKLGVRLV
LGRDKEHPEPKIQRMGDSFAVEIKGRIHFDLFPVVRRTINLPTYTLEAVYEAVLGKTKSKLGAEEIAAIWETEESMK
KLAQYSMEDARATYELGKEFFPMEAELAKLIGQSVWDVSRSSTGNLVEWYLLRVAYARNELAPNKPDEEEYKRRLRT
TYLGGYVKEPEKGLWENIIYLDFRSLYPSIIVTHNVSPDTLEKEGCKNYDVAPIVGYRFCKDFPGFIPSILGDLIAM
RQDIKKKMKSTIDPIEKKMLDYRQRAIKLLANSYYGYMGYPKARWYSKECAESVTAWGRHYIEMTIREIEEKFGFKV
LYADTDGFYATIPGEKPELIKKKAKEFLNYINSKLPGLLELEYEGFYLRGFFVTKKRYAVIDEEGRITTRGLEVVRR
DWSEIAKETQAKVLEAILKEGSVEKAVEVVRDVVEKIAKYRVPLEKLVIHEQITRDLKDYKAIGPHVAIAKRLAARG
IKVKPGTIISYIVLKGSGKISDRVILLTEYDPRKHKYDPDYYIENQVLPAVLRILEAFGYRKEDLRYQSSKQTGLDA
WLKR

>JDF-3 V93R

MILDVDYITENGKPVIRVFKKENGEFRIEYDREFEPYFYALLRDDSAIEEIKKITAERHGRVVKVKRAEKVKKKFLGR SVEVWVLYFTHPQDRPAIRDKIRKHPAVIDIYEYDIPFAKRYLIDKGLIPMEGEEELKLMSFDIETLYHEGEEFGTGP ILMISYADESEARVITWKKIDLPYVEVVSTEKEMIKRFLRVVKEKDPDVLITYNGDNFDFAYLKKRCEKLGVSFTLGR DGSEPKIQRMGDRFAVEVKGRVHFDLYPVIRRTINLPTYTLEAVYEAVFGKPKEKVYAEEIATAWETGEGLERVARYS MEDARVTYELGREFFPMEAQLSRLIGQGLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDERELARRRGGYAGGYVK EPERGLWDNIVYLDFRSLYPSIIITHNVSPDTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMK ATLDPLEKNLLDYRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYADTDGLHA TIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEEGKITTRGLEIVRRDWSEIAKETQA RVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKLVIHEQITRELKDYKATGPHVAIAKRLAARGVKIRPGTVISYI VLKGSGRIGDRAIPFDEFDPTKHKYDADYYIENOVLPAVERILRAFGYRKEDLRYOKTROVGLGAWLKPKGKKK

>JDF-3 V93E

MILDVDYITENGKPVIRVFKKENGEFRIEYDREFEPYFYALLRDDSAIEEIKKITAERHGRVVKVKRAEKVKKKFLGR SVEVWVLYFTHPQDEPAIRDKIRKHPAVIDIYEYDIPFAKRYLIDKGLIPMEGEEELKLMSFDIETLYHEGEEFGTGP ILMISYADESEARVITWKKIDLPYVEVVSTEKEMIKRFLRVVKEKDPDVLITYNGDNFDFAYLKKRCEKLGVSFTLGR DGSEPKIQRMGDRFAVEVKGRVHFDLYPVIRRTINLPTYTLEAVYEAVFGKPKEKVYAEEIATAWETGEGLERVARYS MEDARVTYELGREFFPMEAQLSRLIGQGLWDVSRSSTGNLVEWFLLRKAYERNELAPNKPDERELARRRGGYAGGYVK EPERGLWDNIVYLDFRSLYPSIIITHNVSPDTLNREGCRSYDVAPEVGHKFCKDFPGFIPSLLGNLLEERQKIKRKMK ATLDPLEKNLLDYRQRAIKILANSYYGYYGYARARWYCRECAESVTAWGREYIEMVIRELEEKFGFKVLYADTDGLHA TIPGADAETVKKKAMEFLNYINPKLPGLLELEYEGFYVRGFFVTKKKYAVIDEEGKITTRGLEIVRRDWSEIAKETQA RVLEAILRHGDVEEAVRIVREVTEKLSKYEVPPEKLVIHEQITRELKDYKATGPHVAIAKRLAARGVKIRPGTVISYI VLKGSGRIGDRAIPFDEFDPTKHKYDADYYIENQVLPAVERILRAFGYRKEDLRYQKTRQVGLGAWLKPKGKKK

Fig. 14-1

THE TAG GAG CLA TGT CTG ATG TAT ACT GAG GAT GGA AAG CCC GTC ATC 48

THE TAG GAG CLA TGT CTG ATG TAT TGA CTC CTA CCT TTC GGC CAE TAG 48

M I L D T D Y I T E D G R P V I 16

ACG ATC TTC AAG AAG CAG AAC GGC GAG TTC ACC ATA CAC TAC GAE AGA 56

TCC TAG AAG TTC TTC CTC TTG CGC CTC AAG TGG TAT CTG ATG CTG TCT 96

R I F K K E N G E F T I D Y D R 32

AAC TTT GAG CCA TAC ATC TAC GGC CTC TTG AAG GAC GAC TCT CGG ATT 144

TTG AAA CTC GGT ATG TAG ATG CGC GAG AAC TTC CTC CTG AGA GGC TAA 144

N F E P Y I Y A L L K D D S P I 48

GAG GAC GTC AAG AAG AAA ACT GCC GAG AGG CAC GGC ACT ACC GTT AGG 192

CTC CTG CAG TTC TAC TAC TGA CGG CTC TCC GTC CCG TGA TGG CAA TCC 192

E D V R K I T A E R H G T T V R 64

GTT GTC AGG GCC GAG AAA GTG AAG AAG AAG TTC CTA GGC ACC GCC ATA 240

CAA CAG TCC EGG CTC TTT CAC TTC TTC TTC TAAG GAT CCG TCC GGC TAT 240

CAA CAG TCC EGG CTC TTT CAC TTC TTC TTC TAAG GAT CCG TCC GGC TAT 240

CAA CAG TCC EGG CTC TTC CAC TTC TTC TTC AAG GAT CCG TCC GGC TAT 240

CAA CAG TCC EGG CTC TTC CAC TTC TTC TTC TAAG GAT CCG TCC GGC TAT 240

CAA CAG TCC EGG CTC TTC CAC TTC TTC TTC TTC AAG GAT CCG TCC GGC TAT 240

CAA CAG TCC EGG CTC TTC CAC TTC CTC CCC CAG GAC NNN CCC GCA ATC 268

CTC CAG ACC TTC GAG ATG TAG TTC ACT CAC CCC CAG GAC NNN CCC GCA ATC 268

CTC CAG ACC TTC GAG ATG AAG TGA GTO GGC CTC CTG CGG CCT TAG 288

E V W K L Y F T H P Q D D P A I 96

Fig. 14-2

AGG GAC AAG ATA AAG GAG CAT COT GCC GTT GTG GAC ATC TAC GAG TAC 336 THE CTG THE TAT THE CTC GIA GOA CGG HAR HAD CTG TAG ATG CTC ATG GAC ATO CCC TTC GCG AAG CGC TAC CTC ATA GAC ARA EGC TTA ATC CCG 364 CTG TAG GGG AAG CCC TTC GCG ATG GAG TAT CTG TTT CCG AAT TAG GGC 384 I D K GAL ATG GAG GGC GAG GAA CIT AAG AIG CIC GCC TIC GAC AIC GAG AGG 432 THE CIE CEG CTG CTC CTT GAR TTC THE GAG CGG ANG CTG THE CTC TEC CTC TAT CAC CAG CCC GAG GAG TTC GCC GAA GGG CCT ATC CTG ATG ATA 480 ERG ATA GTG CTC CCG CTC CTC AAG CGG CTT CCC GGA TAG GAC TAC TAT 450 EEFAE 150 AGC TAC CCC GAC CAG GAA GGG GCG CGC GTT ATT ACC TGG AAG AAT ATC 528 TOG ATG CGG CTG CTC CTT CCC CGC GCG CAA TAA TGG ACC TTC TTA TAG T S Y A D GAC CIT CCC TAT GTC GAC GTC GTT TCC ACC GAG AAG GAG ATG ATA AAG . 576 CTE GAR GGG ATA CAG CTG CAG CAA AGG TGG CTC TTC CTC TAC TAT TTC VVSTER CGC TTC CTC AME GTC GTC AME GAN AME GAT CCC GAL GTC CTC ATA ATC GCG AAG GAG TTC CAG CAG TTC CTT TTC CTA GGG CTG CAG CAG TAT TAG 624 RFLKVVK E K D ₽ D V I,

Fig 14-3

THE ARE GGE GAS ARE TTO GAS TTO GGC TAG CIC ARG ARE CGC TOO GAS 672 ATG TIG GGG GTG TTG AAG CTG AAG CGG ATG GAG TTC TIC GGG AGG CTC 672 FAYLKK ARG CTC GGA GTC AAG TTC ATC CTC GGA AGG GAA GGG AGC GRA CCG AAA 720 THE GAG COT CAG THE AAG TAG GAG COT TOO CTT COO TEG CTT GGC TIT 720 240 ATC CAG CGC ATG GGC GAT CGC TIT GCG GTG GAG GTC AAG GGA AGG ATT 768 THE STO GOS THE COS STA GOS AND CGC CAC STO CAS TTO COT TOO TAN 768 E V K G R I 256 CAC THE GAR OFF THE COS STE ATT AGG AGA AGG ATT AAC OFF CCC ACT 816 GTG AAG CTG GAG ATG GGG CAG TAA TCC TCT TGC TAA TTG GAG GGG TGA 816 R THE REE CIT GAG GEA STA TAT GAA GEE ATE TIT GGA DAG DEG AAG DAG ATG TGG GAA CTC CGT CAT ATA CTT CGG TAG AAA CCT GTC GGC TTC CTC AAG GTC TAC CCT CAG GAG ATA GCG CAG GCC TGG GAA ACG GGC GAG GGA BIZ TTC CAG ATG CGA CTC CTC TAT CGC GTC CGG ACC CTT TGC CCG CTC CCT 912 E 304 TTA GAA AGG GTG GCC CGC TAC TCG ATG GAG GAC GCG AAG GTA ACC TAT AAT CIT ICC CAC CCC GCG ATG AGC TAC CTC CTG CGC TTC CAT TGG ATA 960 320

Fig 14-4

GAA CTG GGA AAA GAG TTG TTG CCT ATG GAA CCC CAG CTG TCG CGC CTG 1008 CIT GAG COT TIT CTC AAG AAG GGA TAC CTT CGG GTC GAG AGC GCG GAG GTA GGC CAG AGC CTC TGG GAT GEA TOT CGC TCG AGT ACC CGA AAC CTC 1056 CAT CCG GTC TOG GAG ACC CTA CRT AGA GCG AGC TCA TGG CCT TTG GAG GTC GAG TGG TTT TTG CTG AGG AAG GCC TAC GAG AGG AAT GAA CTT GCA 1104 CAG CTC ACC AAA AAC GAE TCC TTC CGG ATG CTC TCC TTA CIT GAR CGT CCA RAC AAG CCG GAC GAG RGG GAG CTG GCA AGA AGA AGG GAG AGC TAC 1152 GGT TTG TTC GGC CTG CTC TCC CTC GAC CGT TCT TCT TCC CTC TCC ATG R R GCG GGT GGA TAC GTC AAG GAG CCC GAA AGG GGA CTC TGG GAG AAC ATC .1200 CGC CCR CCT ATG CAG TTC CTC GGG CTT TCC CCT GAC ACC CTC TTG TAG STG TAT CTG SAC TIC CGC TCC CTG TAT CCT TCG ATA ATA ATC ACC CAT CAC ATA GAC CTG AAG GCG AGG GAC ATA GGA AGC TAT TAT TAG TGG GTA 1248 ¥ I ARC STO TOO COT GAT ACA CTC ARC AGG GAG GGT TGT GAG GAG TAC GAC 1296 TTE CAG AGE EGA CTA TET GAG TTE TCC CTC CCA ACA CTC CTC ATE CTG

Fig. 14-5

GTG GCT CCT CAG STA GGC CAT AAG TTC TGC AAG GAC TTC CCC GGC TTC CAC CGA GGA GTC CAT CCG GTA TTC AAG ACG TTC CTG AAG GGG CCG AAG 1344 к D F ATC COA AGC CTC CTC GGA GAC CTC TTG GAG FAG AGA CAG AAG GTA AAG 1392 THE SOT TOO GAS GAS COT CTS GAS ARC CTC CTC TCT GTC TTC CAT TTC 1392 AAG AAG ATG AAG GCC ACT ATA GAC CCA ATC GAG AAG AAA CTC CTC GAT ITC ITC TAC TIC CGG TGA TAT CIG GGT TAG CIE TYC TIT GAG GAG CIA 1440 TAC AGG CAA CGA ECA ATC AAA ATC CTT ECT AAT AGC TTC TAC EGT TAC 1488 ATG TCC GTT GCT CGT TAG TTT TAG GAA CGA TTA TCG AAG ATG CCA ATG 1488 ĭ L TAC GGC TAT ACA AAG GCC CGC TGG TAC TAC AAG GAG TGC GCC GAG AGC 1536 ATG DOG ATA TOT TTO DOG GOG ACC ATG ATG TTO DTC ACC DGG CTO TOG 1536 TKARWYYKECAES STE ACC GGT TGG GGC AGG GAG TAC ATC GAG ACC ACG ATA AGG GAA ATA 1584 CAR TGG CCA ACC CCG TCC CTC ATG TAG CTC TGG TGC TAT TCC CTT TAT 1584 Y T R E GAG GAG AAA TIT GGC TIT AAA GTC CTC TAC GGG GAG ACA GAI GGA TIT CTC CTC TTT AAA CCG AAA TTT CAG GAG ATG CGC CTG TGT CTA COT AAA Y TTC GCA AGA ATA CET GGA GCG GAC GCC GAA ACC GTC AAA AAG AAG GCA 1680 AAG CET TET TAT GEA CCT CEC CTG CGG CTT TEG CAG TTT TTC TTC CGT 1680

Fig 14-6

ARG GRE TTC CTG GRE TAG ATC ARC GCC ARA CTG CCC GGC CTG CTC GRA 1728

TTC CTC RAG GRC CTG RIG TAG TTG CGC TTT GRC GGG CCG GRC GRC GRC CTT 1728

K E F L D Y I N A K L L F G L L E 576

CTC GRA TAG GRG GGC TTC TAG RAG CTG GGC GGC TTC TTC GTG ALG ARG ARG 1776

GRG CTT ATG CTC CCG ARG RAG TTC GCG CCG RAG RAG CAC TGC TTC TTC 1776

L E Y E G F Y K R R G F F V K R S G F F V T K K 592

ARG TAC GGC GTT ATR GRC GRG GRG GRG GRG RAG RAG RAG CGC GGC CTT 1824

TTC ATG CGC CRR TAT CTG CTC CTC CTC TTC TAT TGC TGC GGC CGC GAR 1824

K Y A V I D E E D K I T T R G L GGC CGC RAG RAG RAG CGC GGC CTT 1824

GRA ATA CTT RGG CGT GRC TGG RGC GRG RAG RAG RAG RAG RAG RAG CGC GGC GAR 1824

CTT TAT CRA TCC GGR GTG RAC TGG RGC GRG RAG RAG RAG RAG RAG RAG CGC GGC GAR 1824

AGG GTT CTT CRAG GGG RTA CTR RAG CTC TAT CGC TTC CTC TGC GTC CGC 1872

E I V R R D W S E I A K E T Q A G24

AGG GTT CTT CRAG GGG RTA CTR RAG CTC GRC GTC GRC CTT GRA GRA GGG CTA 1920

TCC CRA GRA CTC CGC TAT GRI TTC GTG CCC CAT 1920

R V L E A I L K H G D V E E A V G40

AGG ATT GTC RAR CRAG GTT ACG GRA GAG CTG CAR CTT CTT CGC CAT 1920

R V L E A I L K H G G D V E E A V G40

AGG RTT GTC RAR CRAG GTT ACG CRAG RAG CTG CAR CTT CTT CGC CAT 1920

R V L E A I L K H G D V E E A V G40

AGG RTT GTC RAR CRAG GTT ACG CRAG RAG CTG CAR CTT CTT CGC CAT 1920

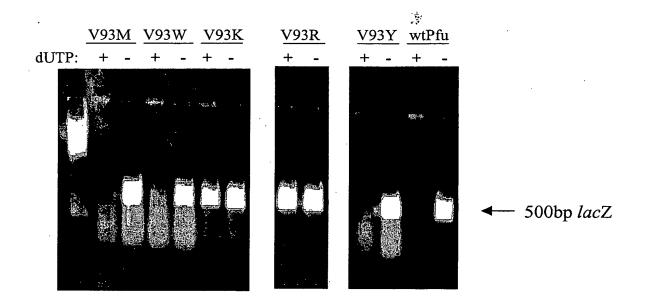
GGG CTT TTC CAG CGC TAT CAT TGC CTC TTC CAC CTG CAR CTT CTT CGC CAT 1920

CCC GRA RAG CTC CGC TAT GRA TGC CTC TTC CAC CAG TTC CTC CAR GGT 1968

R I V K E V T E K L S K L S K Y E V P 656

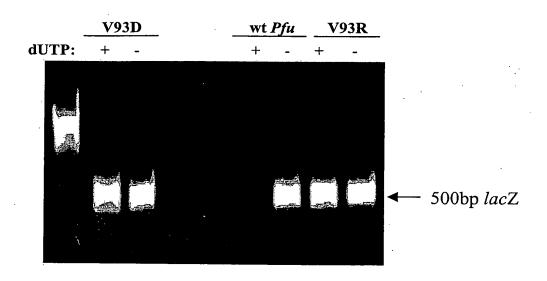
Fig. 14-7

THE ARE SEE ACC SEC CEC CAT STE SET SET SEA ARE ESC CTC SEC SEA 2064 ATG TTC CGG TGG CCC GGC GTA CAC CGA CAA CGT TTT GCG GAG CGG CGT 2064 ATGPHVAVA AGG GGC ATA AAA ATC CGG CCC GGA ACG GTC ATA AGC TAC ATC GTG CTC 2112 THE HEE THE THE GOO GOG COT THE CAR THE THE ATE THE CAC GAG 2112 ARA GGC TCG GGA AGG ATT GGG GAC AGG GCT ATA CCC TTT GAC GAA TTT 2160 TIT CCG AGC CCT TCC TAA CCC CTG TCC CGA TAT GGG AAA CTG CTT AAA 2160 BAC CCG GCA AAG DAC AAG TRU GAT GCA GAA TAC TAC ATC GAG AAC CAG 2208 CTG EGC CGT TTC GTG TTC ATG CTA CGT CTT ATG ATC TAG CTC TTG GTC 2208 E.YYI 736 GTT CTT CCA GCT GTG GAG AGG ATT CTG AGG GCC TTT GGT TAC CGT AAA 2256 CRA GAA GOT GGA CAC CTC TCC TAA GAG TCC CGG AAA CCA ATG GCA TTT 2256 A V E R I L R A F G Y GAA GAT TIA AGG TAT CAG AAA ACG CGG CAG GIT GGC ITG GGG CCG TGG . 2304 CIT CTA MAI TOO ATA GTO TIT TGG GCC GTC CAN COG MAC COO CGC ACC 2304 G A G CTA AAA CCT AAG ACA TGA. 2322 2322 GAT TIT GGR TIC IGT ACT 773



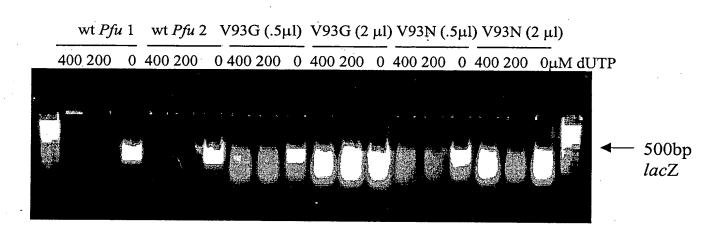
Results: Pfu V93K and V93R mutants show significantly improved dUTP incorporation compared to wild type Pfu. In contrast, the Pfu V93W, V93Y, and V93M mutants show little-to-no improvement in dUTP incorporation.

Figure 15A



Results: The Pfu V93D and V93R mutants show significantly improved dUTP incorporation compared to wild type Pfu.

Figure 15B



Results: The *Pfu* V93N mutant shows a very small improvement in dUTP incorporation compared to wild type *Pfu*. In contrast, the *Pfu* V93G mutant shows little-to-no improvement.

Figure 15C

Figure 16: Polymerase activity and Temperature optimum of Pfu N terminal truncation mutants

Pfu clone #	Truncated after Pfu residue	Relative DNA polymerase activity	Temperature Optimum		
61	H30	Moderate	65°		
72	V66	Similar to wild type	70°		
81	P128	Low	Not tested		
92	I158	Low	Not tested		
3	G125	Similar to wild type	Not tested		
13/14	K201	low	65°		

Pyrococcus furiosus gene for archaeal histone (HMf-like) (Fig. 17-A) (ACCESSION No: AB013081)

K T V K V E D I K L A I K S AAG ACC GTT AAG GTC. GAA GAC ATT AAG CTC GCA ATT AAG AGC K A I E I A K K A V D L A K H A AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA A Q R V S E GCT CAG AGA GTT AGC GAG M M G ATG ATG GGA E L P I A P V D R
GAA TTA CCA ATT GCC CCA GTT GAC AGA Q A A K V CAA GCA GCT AAG GTA L A E H
CTT GCA GAG CAC L I R K
CTT ATA AGA AAG TGA CTT G R GGT AGA GAG GCT × E GAA GGT G 54 162 69 207 36 108 18 54

(HMf-like)-Tag DNA polymerase fusion protein (Fig. 17-B)

AAA GCT AAG ATG ATG 3 ACC GCT ÇAG 3 AGA G E L GGA GAA TTA GIT ATT AAG GAG GIT π Ħ GIC ATC AGC < P I A P V D R L I R CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA GAG GAA GCA Ħ Þ Ħ GAC ATT CAA AAA AAG × A A K V
GCA GCT AAG GTA × AAG GCA GTA × × CIC < GCA GAT Þ U ITA CTT CII ۲ ٢ AAG A GCA GCA GAG Þ S AGC AAG CAC Ħ × CAC H H GCA AAG CIT Þ ᆫ × G R GGT AGA A G GCT GGT GAG 団 GAA 54 162 36 108 69 18 54

// GGC GGC GGT

GTC T S G ACT AGT GGG M ATG CIG 222 Å CIC TTT E GAG 000 P K G AAG GGC CGG GIC V CIC CIG GTG

G H H L A Y R T F H A L K G L T

Н

U

Fig. 178 (cont)

GCC A ရ ရ a GCC CIC D GAC A GCC CIG E GAG SS A GCC S A GCC GAC I ATC CIT GAC 99 R D GAC A GCG CIC CIC I AAG F GGC ; GG G M ATG Y T ACC TTT K AAG 999 9 TAC AAG CGC R R H E A Y G G Y K A G R A CGC CAC GAG GCC TAC GGG GGG TAC AAG GCG GGC CGG GCC E GAG CGG R P P ეეეე Ф GAG CIC CAG A GCG CAC DGAT GCC A A GCC E V
GAG GTC D G D A V I V V GAC GGG GAC GCG GTG ATC GTG GTC N AAC K AAG CIC GAA R Q L A L I CGG CAA CTC GCC CTC ATC P P CIG TGG CIG CIG T ACG CIG CIT K AAG V GTG GCC A R GCG AGG T G D P G AAG D GAC CTT S E GAG Q A X Y G CAG GCG OTC TAC GGC TAC W E K CIC CGG R D GAC ၁၅၅ ၁ CGC ACC TTC K AAG S CTG Y R CGC Y TAC TGG K AAG CIT E GAG I E GAG E GAG A GCG D GAC TCC Y 222 **a** CIG K E L V AAG GAG CTG GTG CAC GTC CAC GCC CTG GAC 9 9 9 CIG A GCC D GAC E GAG GTC CGC R F A K S L R CTG AGG D V GAC GTC N L AAC CTT L H CTC CAC A GCC I ATC E GAG I F D A K A TGG K AAG CGG CIC AAG GTG 222 P 222 d E GAG 999 9 222 Å D GAC 999 CIG T ACC CIC CIC CGC 9 9 9 D GAC CIC 222 P K AAG S E GAG A GCC A GCC CIC ACC CAG AGC CIG OIC A 9 9 D GAC CIG T ACG GAC CIG GAA AAG W TGG Y AAA CIG G G G P CCA 222 A AAG K

Fig. 17B (cont)

a GCC E GAG A GCC မှ မှ Y A GCC CIC CIG TTC CIG R AGG N AAC org V CIG A GCC V CIC R AGG ටටට අ E GAG E GAA A GCC A A R G G R V H R A GCC GCC AGG GGC CGG GCC 960 9 CTG CIG R AGG D GAC CIC TGG W S AGC E G GAA GGC R AGG D GAC CIG TGG W T ACG TTT ეეე ტ CCI P K E A R G AAG GAG GCG CGG GGG 222 A မှ ရှိရှိ E GAG QTG K AAG E GAG GTG CTT CIT R AGG E GAG DOI. CTT A GCC R AGG D GAC GGC CTC N T AAC ACC GIC S CII acce A S TTC CIG CIT R K E CGC AAG GAG AGCT TTC E GAG ପ୍ର ପ୍ର . E GAG GCC E GAG 922 i GIC GGG K R AAA AGG CGC R E GAG T ACC E GAG TTT CIT CIG CTG GAG cee R D P D P A GCC 999 9 L A F CTC GCC A GCC 999 9 222 d GCC E GAG GCC A E GAG R E CGG GAG S AGC р Д GGC CAC R AGG 999 9 D GAC M ATG CIG GCC A W TGG CTC P E K D L AAA GAC CTG M ATG D GAC W A TGG GCC CAC E GAG CIC CIT V GTG ეეე ტ 222 Å E A GAG GCC CCC P D GAC DDD A CIT A GCC P P TCC D L GAT CTT TGG MTG CCT CGG TTC E GAG CGG P P AAC T ACG Cil CGC CIC S AGC Y R AGG E GAA , AAA CIC GTT V 999 9 TAC CIC CIG Y CIC ရှိရှိ ရ မှ မရှင် AGG QTQ A GCC AAC AAC CGG မှ မှ A GCC CIG A GCC I TIC A GCC CII CII

Fg. 17B (cant)

ATC S ရ ရ AAG CIC ၁၅၁ ၁၅၁ N AAC GGC G CGG S AGC S GIC V TCC CGC Q CAG CGC R CIG CGC R GTC V DGAT CGG CIC E GAG T ACG I ATA TTC AAG ეეე 4 A GCC 222 P A GCC E GAG H CAG S T R ACC CGC F TTC N AAC AAG CIC H CIC E GAG T ACC T ACC CIC 222 å Y A GCC မှ မှ မှ R AGG I Y TAC GGC AAG V GTG A GCC Q CAG I ATC R AGG CAG . 9 OTG CGG R TTC I GTC V GTG CIG E GAG N AAC GCC A M ATG D GAC GAC N AAC D GAC CTC TTT GCC ATC E GAG CAG ĐÝĐ B CGC TTC 222 d I ATC ටටට අ ECG S S I ATT .A GCC CIG CAC H ଜନ୍ମ ଜନ୍ମ 222 P T ACG T L K AAG QTC TGG I T ACC M ATG T ACG CTC A GCC 533 F CAC E GAG ncc CTA CGC T ACG D D CIG S AGC E GAG පු ප င်္ကင န CGC TTG T ACC A GCC Y TAC CGG R T ACC 9 9 9 A GCC CIC CAG L CTA CIC Q D E GAC GAG P CCG T ACG A GCC କ ଜନ A GCG A GCC V GTG I Y TAC TTT S S AGC A GCC CIT 9 9 CGG R GTC CAG A GCC CAC CAG TGG N AAC G G G G CIG E GAG CTG E GAG K AAG R AGG 555 P CIG GAC Q CAG M ATG CIC ACC L. CTA AGG Y TIC I R AGG T ACC D P A GCC ATC AGT T ACG

V GTG CCA E GAA T A A D L M K L A M V K L F P R ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC TTC CCC AGG K S V R E A A E R M A F N M P V Q AAG AGC GTG CGG GAG GCC GAG CGC ATG GCC TTC AAC ATG CCC GTC CAG AAG E T GAG ACC K E R AAA GAG AGG M G A ATG GGG GCC GTG CGG GCC TGG ATT CIC R M C AGG ATG A E A V A R L GCG GAG GCC GTG GCC CTG F G R R R Y V L L Q V H
CTC CTT CAG GTC CAC GAG AAG ACC CTG GAG GAG GCC A D GAC P D L E A CCA GAC CTA GAG GCC GAG K AAG GGC AGG E V GAG GTC CIG GIC AGG ATG CIC CGG GAG E GAG CIG ° CGG ₽ GGG 9 6 9 6 မှ မှ မှ - GAG + GTG A GCC TAC

GTG V H CIC H *
CAT TAA Y P L S A K AAG E G GAG GGC A V GCC GTG I D G ATT GAT GGC P L E V CGC R G G GA E GAG ote V ဝ ဝ ဝ 999 9 G H I ATA G E A GGG GAG H H D GAC

CAT

TGG W

Taq DNA polymerase- (HMf-like) fusion protein (Fig. 17-C)

// GGC GGC GGT

T ACT S ପ୍ରତ ପ୍ରତ 222 d TTT E GAG AAG 9 9 CGG R GTC CIC CIG QTG

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FIG 17c (cont)

acc B CIC D GAC GCC CIG E GAG AGC GCC A မေင မေင 33.5 A S GAC GCC A I CIT AAG A GCG GAC CIC R CGG I D GAC TTC GGC CIC ACC Y AAG TTT CGC R K AAG G G G Y CGC R CIC କ୍ଷ କ୍ଷ E GAG E GAG 222 P CAC E GAG P P CAG CGG R A GCG CIC AAG R Q CGG CAA A GCC E WV E. A Y G G Y K A G R A P GAG GCC TAC GGG GGG TAC AAG GCG GGC CGG GCC CCC D G D A V I V GAC GGG GAC GCG GTG ATC GTG ъ Б K AAG A GCC CIC gaa CIG GCC TGG W K AAG V GTG CIG CIG CIT CIG T ACG A GCG T ACC CTT D GAC ncc E GAG 922 P L A L I Q CAG TAC CGC ACC TTC CAC GCC CTG AAG GGC CTC R K HAGG AAG G D GGG GAC W E K TGG GAA AAG GGC TAC CIC D GAC 9 9 A X Y G GCG OTC TAC GGC CGG CGC R TCC CIG Y E GAG ATC E GAG TGG CTT K AAG E GAG TAC K AAG D GAC CCC P TCC A GCG CIG CAC OTC V V F D A K A GTC TTT GAC GCC AAG GCC E GAG CIG A GCC E GAG D GAC OIC A D GAC TTC CGC R L V D A K S A GCC I E GAG N AAC CIG D GAC CIC I CAC CCC CGG TGG R AGG V GTC CIT CIC 222 P G G G E GAG 222 4 T ACC CIG CGC R AAG S AGC 9 9 9 D GAC E GAG A GCC A GCC CIC CIC : ACC 9 9 9 I ATC CAG S AGC CIG ACG ACC CIG GTC D GAC CIC . CCC TGG Y CIG G G G CCA P CIG GAA AAG AAG AAG K AAA AAA

FIG 17 C (cont)

acc A GAG A GCC G G G YAC GCC A CIC CIG TTC CIG R AGG CGC E GAA ၁၁၁ A V GTG N AAC CIG A GCC CGC R E GAG CIC . R AGG GCC CTG QTG -------A A R G G R GCC GCC AGG, GGG GGC CGG R AGG D GAC CIG S AGC TGG CIG CIG TTT E GAA TGG ₩ T ACG ეეე ტ . GGC GIG GCC V GTG 222 A 999 9 K E A R G AAG GAG GCG CGG GGG E GAG CCT AAG GAG CIT GGC CTT D GAC R AGG CIT E GAG S A GCC R AGG CIT A GCG N AAC TTC rcc S R K E
TCC CGC AAG GAG CIG CTT GCC A GCT GAG GGG G T ACC CIC GAG TTC R AGG E GAG K R AAA AGG GTC GTC 999 9 P P CGC R A GCC E GAG T ACC E GAG LLL E GAG CIT HCAC CIG CIG 222 4 222 4 A GCC TIG CGG R ရ ရ P M G CCC ATG R A R E 3 CGG GAG A GCC E GAG CIC A GCC A GCC E GAG 999 9 CC D d S AGC CAC R AGG GAC A GCC 9 9 9 A GCC ය වෙට W TGG CIC P E W A D FIGG GCC GAT K D L AAA GAC CTG HCAC GAG M ATG CIC QTG GAC 222 4 ეეე ჭ CTT ഠ E A GAG GCC CCC CTT DDD P CCG D GAC SS A GCC TTC W TGG ATG CCT CGG E GAG 522 Å CGG R gag T ACG CIC CIT AAC CIT R AGG CGC R S AGC Y TAT E GAA AAA K V GTT CTG GGG Y CIC YAC CIC GCC GTG AAC CGG 9 9 A GCC CIG L GCC A TTC A A CII

Fg 170 (cont)

SAGC I ATC S AGC မှ မှ AAG မ မ CIC AAC CGG CGC R GTC ÇAG CGC R S CIG CGC R DGAT E GAG TTC I ATA R CGG CIC K AAG ଜୁ ଜୁ A GCC ეეე წ E GAG A GCC CAC S AGC TTC GAG CTC N AAC ACC. TACC H Y A GCC မှ မှ R AGG I CIC CGC R Y D D V L GTG CTG A E GCC GAG R V AGG GTC CGG Q CAG TTC I I ATC 255 5 N AAC QTG D GAC D GAC N AAC D GAC M ATG A GCC I ATC ATC E GAG Q CAG E GAG CGC R CIC TTC S D P D P CAC H CAC 9 9 233 d K AAG S A GCC CIG T ACG TTG TGG W OTC I ATC T ACC M ATG ACG CIC A GCC P P CAC E GAG TCC CTA CGC T ACG D GAC CIG S AGC CGC R CGC R CGC ෂ T ACC 999 9 TIG T ACC A GCC CIC Q CAG CGG R CIC ACG A GCC D GAC GTG ъ Э I A GCG Y TAC TTT S. E GAG S AGC A GCC CIT 9 9 CGG R QTC acc A HCAC CAG 999 9 TGG AAC CIG R AGG GAG GAG 222 d CIG D GAC CIC M ATG Q CAG LCTA Y) J A GCC I ATC TTC I R AGO S AGI ACC ACC

Fg. 17 C (cont)

P CCA E GAA T A A D L M K L A M V K L F P R ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC TTC CCC AGG K S V R E A A E R M A F N M P V AAG AGC GTG CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC GTC V E T L
GTG GAG ACC CTC AAG GTG M G A R M ATG GGG GCC AGG ATG AAA E R A TE A V A R L A GAG AGG GCG GAG GCC GTG GCC CGG CTG GCC CGG GCC TGG ATT GAG AAG ACC CTG GAG GAG GGC AGG AGG CGG F G R R R Y V P D L B A L L Q V H D CTC CTT CAG GTC CAC GAC GAG AAG E V GAG GTC L V CIC M ATG GAG CIG CAG CGG E GAG E GAG GCC + GTG

CIC QTG TCC Y TAT ეეე ტ acc A K AAG CIG E GAG A GCC ------V GTG P L I GAT -------E GAG GIG CGC R ۲ GAG 9 9 V G GGA ි ලුලු ල I G E 3 ATA GGG GAG 999 9 H CAT D GAC TGG

H H //
CAT CAT //

GCT ATG ATG Q CAG G E L P I A P V D R L GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT AGA GTT S E Q A A K V L A AGC GAG CAA GCA GCT AAG GTA CTT GCA E H GAG CAC I R K ATA AGA AAG CIT - GAG GCT - GAA GGT 36 108 18 54

I ATT I ATC A K K A GCA AAA AAG GCA V D L A GTA GAT CTT GCA K H AAG CAC GCA GGT AGA 54 162

V E D I K L A I K S *
GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA

AAG

ACC

GIT

AAG

AAA

69

Pfu DNA Polymerase (WT) -(HMf-like) fusion protein (Fig. 17-D)

tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa aataccaatg gagggggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag tgatgtagag aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta aaagattgaa gaagttaaga aaataacggg ggaaaggcat ggaaagattg tgagaattgt taagatagag catgatagaa cttttagacc atacatttac gctcttctca gggatgattc ttacataact gaagaaggaa agcctgttat taggctattc aaaaaagaga acggaaaatt caactctaca cctcccctat tttctctctt atgagatttt taagtatagt tatagagaag tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc

F9 175 (cont)

gaaggagete gaagaaaagt ttggatttaa agteetetae attgacaetg atggteteta ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac atacataaat tcaaagctcc ctggactgct agagcttgaa tatgaagggt tttataagag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc tagagcccta tatccctcga ttataattac ccacaatgtt tctcccgata ctctaaatct aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga

Fig 17D (Cont)

tttgctccaa gcagagccgc tccaatggat aacacccctg ttcccgcacc caagtccgct cagagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct aacttcctgg cttaacatta; aaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa ccagggtaat gtttttaagt atgaaatttt tctttcatag aggaggnnnn nngtcctctc taacttttac agaaataact gtctcaaatt atgacaactc ttgacatttt tacttcatte aaagtataat ttcaagctcc ttcttcttca tgacagatga aaaattgttt tgtctctttt acaatttttt ccttgtatct cctaatgtat aagcaagcca aaggagagta gatgctacct tcaagatttt ctaaaagaat tttaacggcc tcctcgtcaa tttcgacgac gtagatcttt tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatggt tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta aggcgatggt ccaattagca atagggcaat tctagctgag gaatacgatc ccaaaaagca actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca ttccgggagt tttgtattgc tctagccaag gtttgggatt tttgaatcct ttaactctgg tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc

gtagaagcgg cactactcag atgcttcccc aggaatgagg ttgttgtagc tcntcccnga gacactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg gttgagaaaa aagattgaga tgttcttgg // ctcgatttcc ttggttgtgc tccatatgat aagcttccaa agtgggtgtt cagactttta //ATG ATG GGA GAA TTA CCA ATT GCC GCT CAG AGA GTT AGC GAG CAA GCA GCT CCA AAG GIT GTA CTT GCA GAG CAC
GAT CTT GCA AAG CAC
GCA ATT AAG AGC TGA GAC AGA CTT ATA GTA CTT GCA GAG AGA CIT AAG GAG GAA GCT GGT

(HMf-like) - Pfu DNA Polymerase (WT) fusion protein (Fig. 17-E)

AAG ACC AAA GCT

AAG GTC GAA GAC ATT AAG GAG ATC GCA AAA AAG

GCA

GCA

GGT AGA

54 108 162

CIC GTA

AAC	AAA	GCI	ATC
	GCT		
	ATT		
AAG	GAG	GTT	GAA
GTC	ATC	AGC	TTA
GAA	GCA	GAG	CCA
GAC	AAA	CAA	ATT
	AAG		
AAG	GCA	GCT	CCA
CIC	GTA	AAG	GTT
GCA	GAT	GTA	GAC
ATT	CTT	CTT	AGA
AAG	GCA	GCA	CTT
AGC	AAG	GAG	ATA
//	CAC	CAC	AGA
	GCA	CTT	AAG
	GGT	GAG	GCT
	AGA	GAA	GGT
	162	108	54

gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat aaagattgaa gaagttaaga aaataacggg ggaaaggcat ggaaagattg tgagaattgt caactctaca cctcccctat tttctctctt atgagatttt taagtatagt tatagagaag taagatagag catgatagaa cttttagacc atacatttac gctcttctca gggatgattc ttacataact gaagaaggaa aacctgttat taggctattc aaaaaagaga acggaaaatt ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc

FIG 17E (cont)

cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa aataccaatg gagggggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat tagageceta tatecetega trataattae eeacaatgtt tetecegata etetaaatet aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tgatgtagag aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta

Fig. 17 E (cont)

gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa atacataaat tcaaagctcc gtggactgct agagcttgaa tatgaagggt tttataagag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa gaaggagctc gaagaaaagt ttggatttaa agtcctctac attgacactg atggtctcta gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta aacttcctgg cttaacatta aaaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt aggcgatggt ccaattagca atagggcaat tctagctgag gaatacgatc ccaaaaagca actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc

tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatggt ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct cagagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct tcaaggatttt ctaaaagaat tttaacggcc tcctcgtcaa tttcgacgac gtagatcttt ttttgctccaa gcagagccgc tccaatggat aacacccctg ttccgcacc caagtccgct acaatttttt ccttgtatct cctaatgtat aagcaagcca aaggagagt gatgtatct tttaacgccaag gtttgggatt tttgaatcct ttaactctgg aaaagttttac agaaataact ttcttctca tgacaagca aaggaggagt ttttcatcatta ccagggtaat gtttttaagt atgaaatttt tctttcatag aggaggnnnn nngtcctcttt taacttcaaa gttgatgtgt tccaataggtg ctcaatagat aagctccaaa gtgggtgtt cagactttta ggacactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg gttgagaaa gttgagagaga tgttcttgg // TGA

(HMf-like) - PFU DNA POLYMERASE (V93 R OR E) fusion protein (Fig. 17-F)

FIGITE (cont)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

AAA GCT ATT GCT CAG AGA GTT ATG ATG GGA GAA GAG ATC AGC TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA GAG CAA GCA GCT AAG GTA GAA GAC ATT AAG CTC GCA ATT AAG AGC // CTT GCA GAG CAC CIT GCT GAG GAA GGT 108

GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT GAAAAGITIG GATITAAAGI CCTCTACAII 1620 GACACIGAIG GTCTCTAIGC AACTAICCCA GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA CAATGTTTCT ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900 ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG AGTGGAGAGA GGAGACTCAT GTTGAGGTTG CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA, GTTAAGAAAA TAACGGGGGA AAGGCATGGA 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCA: 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA TCGCATTCCC TATCAAGCGA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG TACGATCCCA AAAAGCACAA GTATGACGCA TAAAGCCAGG AATGGTAATT 2100 TGAAGAAGCT 1920 GTGAGAATAG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TACATCGAGT TAGTATGGAA GGAGCTCGAA TCTACGGATA TTATGGCTAT 1500 AGTAGGCCAC 1320 AAGTTCTGCA AGCCCTATAT CCCTCGATTA TAATTACCCA 1740 GAAGGGTTTT ATAAGAGGGG GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG TTAAGACAAA AATGAAGGAA ACTCAAGATC TAACAAGGAC AATAAATCTC TACTTATAAT TCTTCCATAC CGAAGGAGAA CGAGCCCAAG 540

PFU DNA POLYMERASE (V93 R OR E) - (HIMf-like) fusion protein (Fig. 17-G

2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAAG ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 AGGACATCCC 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG ATTCAGÇTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA GAGTTTGGAA AAGATTGTGA GAATTGTTGA TGTAGAGAAG GATAGAACTT TTAGACCATA CATTTACGCT ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 GCTCTAGAAT TIGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ATGCAGAGAA GTTGAGGTTG CTCATCGACA AAGGCCTAAT ACCAATGGAG CAAGATXXXC V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG TATCAAGCGA ;GAGAGAGATG CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA TACGATCCCA AAAAGCACAA GTATGACGCA TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 1740 GAAGGGTTTT ATAAGAGGGG TACATCGAGT TAGTATGGAA GGAGCTCGAA AGCCTGGGAA AATAAATCTC TACTTATAAI CGAAGGAGAA CGAGCCCAAG TCTTCCATAC 720 360

GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA

108 162 //ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT

PFU DNA POLYMERASE (G387P/V93R OR E)-(HMf-like) fusion protein (Fig. 17-II)

CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900 ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT GTTGAGGTTG GCAAAAGCAA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA ${f n}$ GGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACACC ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA GAGTTTGGAA CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATGATTTAG ATGIGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T) GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG GGATACATAG ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TCGCATTCCC TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG TGAAGAAGCT 1920 GTGAGAATAG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 2328 TACGATCCCA TACATCGAGT TAGTATGGAA GGAGCTCGAA TTAAGACAAA AATGAAGGAA ACTCAAGATC AATGGTAATT AACAAGACCA TTACATGAGT TTATGGCTAT 1500 AAGACCTCAG ATACCAAAAG AAAAGCACAA GTATGACGCA CGAGCCCAAG 2100

720

360

180

GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAAAAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA //ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT

(HMf-like) - PFU DNA POLYMERASE (G387P/V93R OR E) fusion protein (Fig. 17-II)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T)

ATG ATG GGA GAA TTA CGA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC //

ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GAGTTIGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CAATGITICI 1260 CCCGATACIC TAAAICTIGA GGGAIGCAAG AACTAIGAIA ICGCICCICA AGIAGGCCAC 1320 AAGIICIGCA ${f N}$ GGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACA<mark>CC</mark> //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG TACATCGAGT TAGTATGGAA GGAGCTCGAA TCTACGGATA TTATGGCTAT 1500 720

GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG ATAAGGCGAT AGGTCCTCAC TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC //TAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 2328 AACAAGACCA TTACATGAGT

(HMF-LIKE)-PFU DNA POLYMERASE (D141A/E143A/V93R OR E) fusion protein

Fig. 17-J)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T)

//ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC // 108

GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA GCTACACAGG.TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA CCAACATACA ATGCAGAGAA GGAGACTCAT GTTGAGGTTG GAGTTTGGAA CTCATCGACA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA AAGATTGTGA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC TCGCATTCCC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA CCACTATTAG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT GAATTGTTGA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GCNATAGCNA CCCTCTATCA AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC TCTTCCATAC AATAAATCTC CGAAGGAGAA CGAGCCCAAG TACTTATAAT 540 360 720

ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC $^{\prime\prime}$ GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG GACACTGATG GACTGCTAGA GICTCTATGC GGGATTTGGA TACAGAAAGG AAGACCTCAG GGAGTTAAAA TAAAGCCAGG AATGGTAATT CTCGCAATAT ATGAGCAGAT AACAAGACCA AGACAAAAGA TTAAGACAAA AATGAAGGAA 2160 TACGATCCCA AAAAGCACAA ACGGAGATGT TGAAGAAGCT 1920 TCATTACTCG GCTTGAATAT 1740 GAAGGGTTTT AACTATCCCA GGAGGAGAAA GTGAGGAAAT 1560 TACATCGAGT TAGTATGGAA GCAAATTCTT TCTACGGATA TTATGGCTAT TGGTTTAGAG ATAGTTAGGA

5

PFU DNA POLYMERASE (D141A/E143A/V93R OR E) -(HMF-LIKE) fusion protein

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T)

GCTACACAGG.TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA CCAACATACA GGAGACTCAT CTCATCGACA CAAGATXXXC AAGATTGTGA ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA GTTGAGGTTG GAGTTTGGAA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG GAATTGTTGA TCGCATTCCC TAGGCGATAT AAGGCCTAAT TATCAAGCGA AAGGCCCAAT CCACTATTAG GACGGCTGTA ATATTTAGCG GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT TATAATGATT ACCAATGGAG TGTAGAGAAG CATTTACGCT AGAAAAAGTT GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATTACTTGGA GCNATAGCNA CCCTCTATCA 1140 CTCAGGGAGA GAGATGGAAG TTCCATTTGC TAACAAGGAC AAAACATAGA AGATAGCAAA AATAAATCTC TCTTCCATAC CGAAGGAGAA AAAGAGATAC AGCCTGGGAA GGAACATCCC CGAGCCCAAG 540 360 720

GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // GAGATTGGAG ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGA! TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG TGAPATTGCA 1860 ABAGAPACTC BAGCTAGAGT TTTGGAGACA ATACTAPAAC 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA TCATTACTCG TGGTTTAGAG ATAGTTAGGA ACGGAGATGT TGAAGAAGCT 1920 GCTTGAATAT 1740 GAAGGGTTTT AACTATCCCA GGAGGAGAAA GTGAGGAAAI 1560 TACATCGAGT TAGTATGGAA

//ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAAAAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA 162 108 5 4

KOD DNA POLYMERASE - (HIMf-like) fusion protein (Fig. 17-L)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

GACATTGAAA AAGCGCTGTG GTTGACGTCG AGCTACGCCG CTCATAGACA CGAGAGCATC GAGGTCTGGA AACTCTACTT TACTCATCCG ACGGTTGTAA CGGTTAAGCG CTCCTGAAGG AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT ATGATCCTCG ACACTGACTA CATAACCGAG V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) TATCCTGTGA TAAGACGGAC GATAAACCTG AGGGATTAGT GCCAATGGAA CAGCAGTTAT TGACATCTAC ACGATTCTGC TGGGCGACAG GTTTGCCGTC ACGTTCTCAT TCTCGACGGA GAGGGAGATG ACGAGGAAGG GGCCAGGGTG CTCTCTACCA TGAGGGCGAG AAAAGCTCGG AACCTACAAC AATAAACTTC GGTTGAAAAG CATTGAGGAA CCCACATACA GGCGACAACT ATAAAGCGCT ATAACTTGGA GAGTTCGCCG GGCGACGAGG GAGTACGACA CAGGACXXXC GTCAAGAAGA GATGGAAAGC GAAGTGAAGG GCCCTCGGAA GTTCAGAAGA CGCTTGAGGC CGTTTATGAA GACGGATACA GGGATGGAAG TCGACTTCGC TCCTCCGTGT AGAACGTGGA AGGGGCCAAT CCTTATGATA AGCTGAAAAT GCTCGCCTTC TACCCTTCGC CAAGCGCTAC CAGCGATAAG AGTICCTCGG GAGACCAGTI TTGAACCCTA CTTCTACGCC CTGTCATAAG AATTTTCAAG TAACCGCCGA GAGGCACGGG TGTGAAGGAG TCTCCCCTAC CGAGCCGAAG GGACAAGATA CTTCGATCTC CTATCTGAAA 660 600 540 480 420 120 360 300 180

GTTGAGAGAA TTCTGAGAGC CTTCGGTTAC CGCAAGGAAG ACCTGCGCTA CCAGAAGACG 2280 GACCCGACGA AGCACAAGTA GAGACGCAGG GGCAAGATAA AGACAGGTIG GITTGAGTGC TIGGCTGAAG CCGAAGGGAA CT 2325 TACATCGTGC TCAAGGGCTC TGGGAGGATA GGCGACAGGG CGATACCGTT GCCGTTGCCA GTGATCCACG AGCAGATAAC AGGATAGTCA GGCTTCTACA ATGGAGTTCC ACCGACGGAT ATAACGATGA TACAGGCAGA CAGAAGATAA TTCTGCAAGG GTGTACCTAG CGGCAGAGCT GCCGTCTTCG GTCAGCCGAA AGGGCGCGCT GATACGCTCA GCCTATGAGA CTCTGGGACG ACCGGCGAGA ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAP AGAGGTTGGC AAGAAGTTAC CGAGGGTTCT CAACGCGCGG GGGCCATCAA ACCTTGAGAG AACGCGGCTT TCAAGTATAT CCATCAAGGA GGTACTGCAA AGAAGAAGAT ACTICCCAGG ATGAAGGAGG AGGAGTTCCT TTTTTGCCAC ACAGAGAAGG ATTTTAGATC GGAATGAGCT TCTCCCGCTC CGACGCCGAG CGCGAGAGGA GTCAAAATAC GCCCTGGAAC GAGGGATTTA AAGGACTACA AGGCAACCGG CGAAAAGCTG ACTTGAGATT CTTCGTCACG CAACGCCAAA GATAGAGGAA AAGTACGGCT GGAGTGTGCA GATCCTGGCA GAAGGCCACG GGAGAAGGTT TACGCTGAGG TGAAGCTTTG ATTTATCCCG ATGCAAGGAA AATACCTGGA CTATGTAAAA GGCCCCGAAC AGTCGCCCGC CCTGTACCCC CAGCACTGGC TCCGATGGAG TACTACATTG AGAACCAGGT TCTCCCAGCC CTTCCGGGCG GCCGATGCTG GAGAGCGTAA AACAGCTACT TATGACGTTG TCAATCATCA GAGCCCGAGA AGCAAGTACG CTAAAGGACG GTGAGGCGTG AAGAAGAAGT ATTGACCCGA AGCCTGCTTG AAGCCCGATG GCCCAGCTTT TACTCGATGG AACCTCGTTG AGGTTCCGCC GTGACGTCGA ACTGGAGCGA ATGCGGTGAT CGCTTGAGCT AAACCGTCAA TTAAGGTAAT CGGCCTGGGG ACGGTTACTA TCGAGAGGAA GAGACCTCCT CCCCACAGGT TCACCCACAA GAGGGTTGTG AAAAGGAGCT AGTGGTTCCT CTCGCTTAAT AAGATGCGAA AAATAACCAC AGCCTGGGAA CGACGAGTTC GATAGCGAAA AGACGAGGAA AAAGAAGGCT GGTCACATAC GGTGATAAGC GAAGGCCGTG CGAGTACGAG CTACAGCGAC GCTCCTCGAT AGAGGAGAGG TCCCCACGIT GGAGAAGCTG CGGCTATGCA CGGCCACCGC CGTCTCGCCG GGAGAACATA CGGCCAGTCC AAGGGAGTAC GGCCAGAAGA CCTCAGGAAG 2040 2100 1860 1380 2160 1980 1800 1740 1680 1620 1140 1920 1560 1500 1440 1260 1200 1080 1020 1320 108

(HMf-like) - KOD DNA POLYMERASE fusion protein (Fig. 17-M)

V93E MUTANT: V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

//ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT

54

ITM (cont,

GCT AAA

GCT CAG

ATT GAG ATC AGA GTT AGC

GCA GAA GAC

GAG CAA GCA AAA

GCT AAG GTA CTT GCA GAG CAC

GTT

AAG GTC

ATT AAG

AAG CTC GCA ATT AAG AGC

GCA GTA GAT CTT

GCA AAG

CAC GCA GGT CTT

AGA GAA

162 108

GAG

CAGAAGATAA CGGCAGAGCT GCCTATGAGA GAGCTTGGGA ACCGGCGAGA GCCGTCTTCG AAAGACCCGG GTTGACGTCG TCTCGACGGA AGCTACGCCG ACGAGGAAGG GAGACGCAGG GGCAAGATAA ATGGAGTTCC ACCGACGGAT ATAACGATGA GATACGCTCA GTGTACCTAG CTCTGGGACG ATTCAGAGGA AAGCGCTGTG CGAGAGCATC ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA AGGATAGTCA GGCTTCTACA AGGGCGCGCT TACAGGCAGA TATCCTGTGA CTCATAGACA AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT TTGAACCCTA CTTCTACGCC 120 ITCTGCAAGG GAGGTCTGGA CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA //ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC CTGTCATAAG AATTTTCAAG 60 AACTCTACTT TACTCATCCG CAACGCGCGG GGGCCATCAA ATGAAGGAGG GGAATGAGCT TCTCCCGCTC AGGAGTTCCT GTCAGCCGAA TAAGACGGAC TGGGCGACAG AAAAGCTCGG ACGITCTCAT CTCTCTACCA AGGGATTAGT CAGCAGTTAT TGACATCTAC GAGTACGACA AAGAAGTTAC CGAGGGTTCT AACGCGGCTT TCAAGTATAT CCATCAAGGA GGTACTGCAA AGAAGAAGAT ACAGAGAAGG ATTTTAGATC ACCTTGAGAG TTTTTGCCAC ACTTCCCAGG GATCCTGGCA CGAAAAGCTG TGAAGCTTTG ACTTGAGATT CTTCGTCACG CAACGCCAAA AATACCTGGA GATAGAGGAA GGAGTGTGCA GAAGGCCACG ATTTATCCCG ATGCAAGGAA CCTGTACCCC CTATGTAAAA GGCCCCGAAC CAGCACTGGC TCCGATGGAG AGTCGCCCGC GGAGAAGGTT GATAAACCTG GTTTGCCGTC AATAAACTTC AACCTACAAC GAGGGAGATG GGCCAGGGTG TGAGGGCGAG GCCAATGGAA GCCGATGCTG AAGTACGGCT GAGAGCGTAA GGCGACGAGG CAGGACXXXC GTGAGGCGTG AAGAAGAAGT CTTCCGGGCG AACAGCTACT ATTGACCCGA GAGCCCGAGA AAGCCCGATG GCCCAGCTTT GAAGTGAAGG GCCCTCGGAA AGCCTGCTTG TATGACGTTG TACGCTGAGG CCCACATACA GGCGACAACT ATAAAGCGCT ATAACTTGGA GAGTTCGCCG AGCAAGTACG CTAAAGGACG TCAATCATCA AACCTCGTTG TACTCGATGG AGTTCCTCGG GAGACCAGTT ACTGGAGCGA ATGCGGTGAT CGCTTGAGCT AAACCGTCAA CGGCCTGGGG ACGGTTACTA GAGACCTCCT CCCCACAGGT TCACCCACAA GAGGGTTGTG AAAAGGAGCT AGTGGTTCCT CTCGCTTAAT AAATAACCAC GACGGATACA TCGACTTCGC TCCTCCGTGT AGGGGCCAAT TACCCTTCGC CAGCGATAAG TAACCGCCGA AGGTTCCGCC GTGACGTCGA TTAAGGTAAT TCGAGAGGAA AAGATGCGAA CGCTTGAGGC GGGATGGAAG AGAACGTGGA AGCTGAAAAT GCTCCTCGAT GAGGCACGGG GGAGAAGCTG GATAGCGAAA AGACGAGGAA CGAGTACGAG CTACAGCGAC CGGCTATGCA CGTCTCGCCG GGAGAACATA GGCCAGAAGA CGGCCAGTCC GAAGGCCGTG AAAGAAGGCT AAGGGAGTAC AGAGGAGAGG CGGCCACCGC CCTCAGGAAG GGTCACATAC AGCCTGGGAA CGTTTATGAA CTTCGATCTC CGAGCCGAAG CTATCTGAAA TGTGAAGGAG TCTCCCCTAC CCTTATGATA GCTCGCCTTC CAAGCGCTAC GGACAAGATA 1440 1320 1260 1140 1020 960 900 840 780 720 660 600 300 240 1860 1740 1680 1620 1560 1380 1200 1080 1800 1980 150C 192(

GTGATCCACG

AGCAGATAAC GAGGGATTTA AAGGACTACA AGGCAACCGG

TCCCCACGTT

AGACAGGTTG GTTTGAGTGC TTGGCTGAAG CCGAAGGGAA CT //TAG 2325 GACCCGACGA AGCACAAGTA CGACGCCGAG TACTACATTG AGAACCAGGT TCTCCCAGCC TACATCGTGC TCAAGGGCTC GCCGTTGCCA AGAGGTTGGC CGCGAGAGGA GTCAAAATAC GCCCTGGAAC GGTGATAAGC GTTGAGAGAA TTCTGAGAGC TGGGAGGATA GGCGACAGGG CGATACCGTT CGACGAGTTC CTTCGGTTAC CGCAAGGAAG ACCTGCGCTA CCAGAAGACG 2100 2220 2160

HMf-like) - Vent DNA POLYMERASE FUSION PROTEIN (Fig. 17-N)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) //ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC // 108

CAAAGTGTAT ACGTATGAGC GATCTTTTCC CCCAAGATTC GTCGATGTTG AGTTATGCCG GATATTGAAA AGGGAACATC AGGGTGGCAT ACGCGAGGAA TGGGAAACAG TATGAAGCAG AAACGGGCAG AAAGACCCCG GAAGTCTGGA AAAACTGTGA AAAGAGAACG ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CGGCGCTTAA GAACAACTTA CCTGGGAGGA CTCATAGACA CTTCTCAAAG GGGACGTCTC AAGAAAGCAT AAAAGCTGGG GAGTGCTCGA GGGAGTTTAA AATAGAACTT GACCCTCATT TCGGGAAGGA TTTTAGGAAA AACCAAAAGC AAATTAGGAG CAGTTGTGCG AGAGGATGGG ATGTGATAAT TGTCCAATGA ATGAAGAAGA CGTTTTATCA AGGGCTTGAT CAGCTGTGGT AGCTCATTTT ATGACTCCGC TGAACTTGCA GAGATCAAGC ATTCTTCCCC GAAAAAACTA AAGGACGATA TGATAGTTTT AGTTCGGCTT AACTTACAAT AAGAGAAATG GGCCAGAGTA TGAGGGAGAT TCCCATGGAG TGACATTTAC CGAGCATCCC TGCAGTGAAA TATTGAGGAG TATGTAAAAG GCTGTGGAAA ACCGGCAACC ATGGAAGCTG GCCCAGTACT GAATTTGGAA CCGAACAAAC AACCTCCCAA GTCTTAGGAA GTCAGGAAAA ATAAAGGCAA GGGGACAATT ATAAAGCGTT ATCACATGGA GGAGACGAGG GAATATGACA CAAGACXXXC AGCCAGAAAA TCGTGGAGTG AGCTGGCAAA CAATGGAAGA CAGAGGAAAT CGTATACGCT TCAAGGGTAG GGGACAAAGA AGGGCGAGAT TACCCTTTGC CAGCTATGCG AATTTTTGGG TAAAGGGCGA CTATAATCCG CTGATGAGGA TTGATTTGCC AAAATATCGA AGCTTAAGCT TTCAGCCCTA TIGTICAAGI AGGTTTGTGG GCTGATAGGT AGAGTATAAA GTATCTTTTA TGCTAGGGCA TGCCGCTATA TGAGGCAGTT AATCCACTTT ACATCCCGAA GTATCTCATA TGTTAAAGAA TTTGCCGTAT AATAATGATT CCTTGCCTTT CAAGCGTTAT GGGCAAAATA GAGACATGGA TATATATGCT AATTTTTAAG AAGGGAAGTT 960 900 840 660 600 540 480 420 1080 1020 780 360 300 120 240 180

ACAGAATACG CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GGTAG 2325 TTGCCGGCAG ATAATAAGCT CCTCATGTCG GAAAAGCTTG AAAGCTGTAG ATAGCTAAGG GATGAAGAGG GAGTATGAGG AAGAAAGCCA GGGTATCCTA GCAATGAGGC GGATATAGGT GTATCCCCAG ATGCTCGATT TATGCGGACA AGACACTACA ATAGGCAAAG TTTATTTGGA TACTTAGGAT ATCCTAGAAA ATATCGTTCT CGATAGCAAA TTATCCATGA AAGTTGTTAG AGACTCAGGC GCAGGATAAC GCTTTTACTT AGGAATTCCT CTGACGGCTT TAGAGATGAC AGGCAAGATG AAGATATAAA TCTGCAAGGA ATACCCTTGA ACTCGAAGCG GCAGATTACC CAAAGGGAGC AAGACTTGCC GAGAGGATTC ACACAAGTAC AAACTACATA TTATGCCACA GGCTATTAAA GAAGAAAATG TTTCCGCAGT AGATGTTGTA AAAGGTTTTA AACAAGGGGC GTACTCGAAG CTTTCCGGGC AAAAGAGGGC GATAAGAGAA TTTGGATACA GAAAGGAGGA GATCCGGACT ACTACATAGA AAACCAAGTT GGAAAGATAA GCAAGAGGGA TAAAAGTGAA GAGAAAATAG GAGGCTATAC AACTCCAAAC ATACCCGGGG ATAGAGGAAA AGTTCGGCTT GAATGTGCTG AGGGATTTAA TTGGAAGTAG CTGTACCCTT TTTGTTACAA AAAAGCGCTA TTGCTTGCAA AAATCCACAA TTTATTCCCT TGTAAGAATT GCGATAGGGT AGGACTACAA CAAAATACAG TAAGGAGAGA TTCCAGGTCT AAAAGCCTGA AAAGCGTTAC ACAGCTATTA TTAAAGAGGG TTGACCCGAT CCATACTCGG ACGATGTTGC CAATAATAGT AATTTTACTT ACCGGGCACA AGCCATTGGC TTTAAGGTAT GCTTGAGCTT GGTTCCACTT AAGTGTTGAA TGCAGTCATA ACTCATTAAA TAAGGTTCTT CGGCTATATG CGAAAAGAAA GGACTTAATT TCCGATAGTA TTGGAGTGAG CGCATGGGGG TACTCACAAC 1860 2220 1800 1740 2160 2100 2040 1980 1920 1680 1620 1260 1560 1500 144C

Vent DNA POLYMERASE - (HMf-like) FUSION PROTEIN (Fig. 17-0)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

AGTTATGCCG AGGGAACATC ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC GTCGATGTTG GATATTGAAA CTCATAGACA GAAGTCTGGA AAAACTGTGA CTTCTCAAAG AAAGAGAACG AAAGACCCCG ATGTGATAAT AACTTACAAT GGGGACAATT TTGATTTGCC CGTTTTATCA AGGGCTTGAT CAGCTGTGGT GAGTGCTCGA ATGACTCCGC TGTCCAATGA ATGAAGAAGA AGCTCATTTT GGGAGTTTAA GGCCAGAGTA TGACATTTAC CGAGCATCCC TATTGAGGAG AATAGAACTT AAGAGAAATG TGAGGGAGAT TCCCATGGAG TGCAGTGAAA ATAAAGCGTT GAATATGACA GTCAGGAAAA ATAAAGGCAA GACCCTCATT ATCACATGGA GAATTTGGAA GGAGACGAGG CAAGACXXXC AATTTTTGGG TAAAGGGCGA TTGTTCAAGT AAAATATCGA AGGGCGAGAT AGCTTAAGCT TACCCTTTGC CAGCTATGCG TTCAGCCCTA CTATAATCCG AATTTTTAAG GTATCTCATA AATAATGATT GGGCAAAATA GAGACATGGA TATATATGCT CCTTGCCTTT CAAGCGTTAT AAGGGAAGTT TGTTAAAGAA TTTGCCGTAT 480 420 360 120 600 540 300 240 180

ATAGCTAAGG CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GG 2325 // ACAGAATACG ATAATAAGCT CCTCATGTCG GAAAAGCTTG AAAGCTGTAG GATGAAGAGG GAGTATGAGG AAGAAAGCCA TATGCGGACA AGACACTACA GGGTATCCTA ATGCTCGATT ATAGGCAAAG GCAATGAGGC GGATATAGGT TCTGCAAGGA GTATCCCCAG GAAAATATCA CGGCGCTTAA AGGGTGGCAT CAAAGTGTAT GATCTTTTCC ACGTATGAGC TGGGAAACAG AAGAAAGCAT GAAAAAACTA TIGCCGGCAG TATGAAGCAG AAACGGGCAG AAAAGCTGGG AGACTCAGGC GCAGGATAAC GCTTTTACTT AAGATATAAA TACTTAGGAT ATCCTAGAAA ATATCGTTCT CGATAGCAAA TTATCCATGA AAGTTGTTAG AGGAATTCCT CTGACGGCTT TAGAGATGAC AGGCAAGATG ATACCCTTGA TTTATTTGGA GAACAACTTA ACGCGAGGAA GGGACGTCTC AGAGGATGGG CAGTTGTGCG TCGGGAAGGA TTTTAGGAAA ACACAAGTAC GATCCGGACT ACTACATAGA GAGAGGATTC GAAGAAAATG ACTCGAAGCG TTTGGATACA GAAAGGAGGA CAAAGGGAGC AAGACTTGCC GCAGATTACC AGATGTTGTA AAAGGTTTTA AACAAGGGGC AAACTACATA TTATGCCACA GATAAGAGAA GTACTCGAAG GGCTATTAAA CTTTCCGGGC AAAAGAGGGC CCTGGGAGGA GAGATCAAGC ATTCTTCCCC AACCAAAAGC AAGGACGATA AGTTCGGCTT TTTCCGCAGT TGAACTTGCA TGATAGTTTT GAGGCTATAC GGAAAGATAA GCAAGAGGGA AGGGATTTAA AGGACTACAA GAGAAAATAG TTGCTTGCAA GCTGTGGAAA TCAAGGGTAG GTCTTAGGAA GGGACAAAGA ACATCCCGAA TTGGAAGTAG AACTCCAAAC ATACCCGGGG ATAGAGGAAA GAATGTGCTG AAATCCACAA TATGTAAAAG CCGAACAAAC AACCTCCCAA CGTATACGCT TTTGTTACAA TTTATTCCCT TGTAAGAATT CTGTACCCTT ACCGGCAACC ATGGAAGCTG GCCCAGTACT AAATTAGGAG GCGATAGGGT CAAAATACAG AAAAGCGCTA TAAAAGTGAA TTAAAGAGGG TAAGGAGAGA TTCCAGGTCT AAAAGCCTGA AGTTCGGCTT AAAGCGTTAC ACAGCTATTA TTGACCCGAT CCATACTCGG ACGATGTTGC CAATAATAGT AGCCAGAAAA CTGATGAGGA TCGTGGAGTG AGCTGGCAAA CAATGGAAGA CAGAGGAAAT TGCAGTCATA GCTTGAGCTT AAACCAAGTT AATTTTACTT ACCGGGCACA AGCCATTGGC GGTTCCACTT TGCTAGGGCA TGCCGCTATA TGAGGCAGTT 840 TTGGAGTGAG ACTCATTAAA TAAGGTTCTT CGGCTATATG CGAAAAGAAA GGACTTAATT TCCGATAGTA AGGTTTGTGG AGAGTATAAA GTATCTTTTA GCTGATAGGT AATCCACTTT TTTAAGGTAT AAGTGTTGAA CGCATGGGGG TACTCACAAC 1800 900 2160 2040 1980 1920 1860 1740 1680 1620 1500 1440 1380 1320 1260 1140 1080 1020 960 2100 1560 1200

//ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAI AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGI AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA 108 162

Deep Vent- (HMf-like) DNA polymerase fusion protein (Fig. 17-P)

Fig 17P (cont)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)
V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

2160	TGCAGAGGAG	GGGCTATCCT	ATAAGCAAGA	AGACGGGCCA	TGCTGAGGGG	GGGTACATAG
ָ ה כר		TOPOCOTION OF THE PROPERTY OF	CCACTA A ACC	ADCIDION A	CANANCETT	CHACTCALA
04	AGGTCCGCAC	ACAAGGCTAT	CTTCACGAGT	CACGAGGCCC	ACGAGCAGAT	CTAGTTATTT
86	TCCAGAAAAG	ACGAAATACC	CTGAGCAAGT	AACTGAAAAG	TTAAGGAGGT	GTAAAGATAG
1920	TGAGGAGGCA	ATGGCAACGT	ATCCTAAAGC	CCTAGAGGCT	AAGCAAAAGT	AAAGAAACCC
σ	CGAAATAGCC	GGGACTGGAG	ATAGTCAGGA	GGGGCTTGAA	TAATCACTAG	GAAGGGAAGA
1800	GATAGATGAG	AGTATGCGTT	ACGAAGAAGA	GTTCTTCGTG	ACGTGAGAGG	GAGGGCTTCT
1740	GCTTGAGTAC	GGCTGTTGGA	AAGCTCCCAG	TATAAACGCC	TCGTAGATTA	GCCCTAGAGT
1680	AAAGAAGAAA	CCGAGGAGAT	GGGCAAAAC	CACAATTCCT	GACTCTACGC	GACACAGATG
1620	CTTATACATA	GGTTCAAAGT	GAAAAGTTCG	GGAACTGGAG	TCGTAAGGAA	TATATAGAGT
1560	GGGGAGGGAA	TTACGGCCTG	GCAGAGAGCG	TAAGGAGTGC	GTTGGTACTG	GCAAAAGCCC
1500	TTATGGGTAC	ATTATGGGTA	GCAAACAGCT	CAAAATCCTG	AACGGGCAAT	GATTACAGGC
1440	GAAGATGCTT	CAATCGAGAA	TCTAAAGACC	GATGAAAGCT	TAAAAAGGAA	AGGCAAGAAA
1380	ATTGGATGAA	TCAAGAGGTT	CCCAGCCTGC	GGGGTTTATC	AGGACTTCCC	AAGTTCTGCA
1320	GGTTGGGCAC	TCGCCCCAGA	GAATACGATG	AGGGTGTAGG	TGAACAGGGA	CCGGATACGC
1260	TAACGTCTCA	TAATCACCCA	CCCTCGATAA	GAGCCTGTAC	TAGATTTCAG	TTAGTTTCCC
1200	CTGGGAGGGG	AGAAAGGGCT	AAGGAGCCGG	GGGATACGTT	GCTACGCTGG	CTAAGGGAGA
1140	CGAGAGAAGG	AGAGGGAGTA	AAGCCGGATG	GGCTCCAAAC	GGAATGAATT	GCCTACGAGA
1080	CCTCAGGAAG	AGTGGTACCT	AACTTGGTGG	TTCAACTGGC	TTTCTAGGTC	CTGTGGGATG
1020	CGGCCAGCCC	CAAGGTTAGT	GCCCAGCTTT	CCCAATGGAG	GGGAGTTCTT	GAGCTCGGTA
960	GGTAACGTAC	AGGATGCAAA	TATTCAATGG	AGTTGCAAAG	GACTGGAGAG	ACTGGAAAGG
900	GGCCTGGGAG	AGATAGCTGA	TACGCTCACG	GGAGAAAGTT	GAAAGCCAAA	GCAATCTTCG
840	AGTTTATGAG	CCCTCGAGGC	CCAACATACA	GATAAACCTC	TTAGGAGAAC	TACCACGTGA
780	CTTTGACCTC	GAAGGATACA	GAGATAAAGG	GACAGCGGTG	TTGGGGATAT	ATGCAGAGGC
720	TGAGCCAAAG	GGGACGGTAG	CCCCTGGGAA	GATAAAGCTA	AAAAGCTCGG	AAGAGGGCCG
660	CTATCTAGTT	TCGACCTTCC	GGCGATTCTT	TACCTACAAC	ATGTTATAAT	AAAGATCCCG
600	GATAAGGGAG	TCCTCAAGGT	ATAAAGCGGT	GAGGGAGATG	TTTCCAGCGA	GTCGAGGTAG
540	TCTCCCGTAC	AAAAGATCGA	ATAACGTGGA	AGCCAAAGTC	ATGAGGAAGA	AGCTATGCTG
480	TATAATGATA	AGGGGCCCAT	GAGTTCGCGA	CGAAGGGGAG	CCCTCTATCA	GACATAGAAA
N	GCTCGCATTT	AGCTCAAGTT	GGCGATGAAG	TCCAATGGAA	AAGGCCTAAT	CTAATAGACA
360	GAAGAGGTAC	TTCCGTTCGC	GAGTACGACA	TGACATCTTT	CCGCAGTTAT	AGAGAGCATT
0	GGATAAGATA	CCGCAATAAG	CAGGACXXXC	TGAACACCCT	GGCTGTACTT	GAGGTATGGA
240	GAGGCCGATT	AGTTCCTGGG	GTAAGGAAGA	TGCCGAAAAG	GAATTATAGA	AAGATAGTGA
σ	GAGGCATGGG	TAACCGCCGA	GTTAGGAAGA	GATTGATGAG	ATGACTCGCA	CTCCTCAAAG
120	CATTTACGCT	TTAGACCTTA	GACAGAAACT	GGTTGAGTAC	GCGAGTTTAA	AAAGAAAACG
60	GATTTTCAAG	CGATTATAAG	GATGGGAAGC	CATCACCGAG	ACGCTGACTA	ATGATACTTG

ACTAAACAGA CAGGTCTTAC GGCATGGCTT AACATCAAGA AGAAG // GCCGTTCTTA GAATATTAGA GGCCTTTGGG TACAGGAAAG AAGACCTCAG GTGGCAGAAG TTCGATCTCA GGAAGCATAA GTATGACGCT GAGTATTACA TAGAAAATCA GGTTTTACCT //AIG AIG GGA GAA TIA CCA AIT GCC CCA GII GAC AGA CII AIA AGA AAG GCI GGI AAG ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC TGA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA 2280 2220 108

(HMflike) - Deep Vent DNA polymerase fusion protein (Fig. 17-Q)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE

//ATG ATG GCT CAG AGA GTT AGC AAG ACC GTT AAG GTC AAA GCT ATT GAG ATC GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GAA GAC ATT AAG GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GAG CAA GCA GCT AAG GTA CTT GCA GAG CTC GCA ATT AAG AGC CAC TGA GCA GGT AGA CTT GAG GAA GGT 108

GCAATCTTCG ATGCAGAGGC AAAGATCCCG AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGTACGACA AGCTATGCTG AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA AGTTCCTGGG GAGGCCGATT ACTGGAAAGG TACCACGTGA AAGAGGGCCG GICGAGGIAG GACATAGAAA GAGGTATGGA GGCTGTACTT CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGG AAAGAAAACG GCGAGTTTAA GGTTGAGTAC GACAGAAACT TTAGACCTTA CATTTACGCT CTAATAGACA //ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG GACTGGAGAG AGTTGCAAAG GAAAGCCAAA TTGGGGATAT AAAAGCTCGG ATGTTATAAT TACCTACAAC ATGAGGAAGA CCCTCTATCA AAGGCCTAAT TTAGGAGAAC TTTCCAGCGA GAGGGAGATG GATAAACCTC GGAGAAAGTT GACAGCGGTG GATAAAGCTA AGCCAAAGTC CGAAGGGGAG TCCAATGGAA TGAACACCCT CAGGACXXXC GGCGATTCTT GGCGATGAAG GAGATAAAGG CCCCTGGGAA TATTCAATGG TACGCTCACG ATAAAGCGGT CCAACATACA ATAACGTGGA GAGTTCGCGA AGGATGCAAA GGTAACGTAC AGATAGCTGA GGCCTGGGAG CCCTCGAGGC GAAGGATACA GGGACGGTAG TCGACCTTCC AGGGGCCCAT AGCTCAAGTT TTCCGTTCGC CCGCAATAAG GGATAAGATA TCCTCAAGGT AAAAGATCGA AGTTTATGAG GAAGAGGTAC CTTTGACCTC TGAGCCAAAG CTATCTAGTT GATAAGGGAG TCTCCCGTAC TATAATGATA GCTCGCATTI 720 660 600 480 360 300 240 180

GTAAAGATAG ACTAAACAGA GCCGTTCTTA GGGTACATAG GTTGCCGTGG CTAGTTATTT AAAGAAACCC GAAGGGAAGA GAGGGCTTCT GCCCTAGAGT GACACAGATG GCAAAAGCCC GATTACAGGC AAGTTCTGCA CCGGATACGC TTAGTTTCCC GCCTACGAGA TATATAGAGT AGGCAAGAAA CTAAGGGAGA CTGTGGGATG GAGCTCGGTA CAGGTCTTAC GAATATTAGA GGGAGTTCTT GGAAGCATAA ACGAGCAGAT CAAAAAGGTT TAATCACTAG ACGTGAGAGG GACTCTACGC TGCTGAGGGG TTAAGGAGGT TCGTAGATTA TCGTAAGGAA GTTGGTACTG TAAAAAGGAA AGGACTTCCC TAGATTTCAG GGAATGAATT AAGCAAAAGT AACGGGCAAT TGAACAGGGA GCTACGCTGG TTTCTAGGTC GGCATGGCTT GGCCTTTGGG GTATGACGCT AGACGGGCCA AGCCGCTAGA CACGAGGCCC AACTGAAAAG CCTAGAGGCT GGGGCTTGAA GTTCTTCGTG CACAATTCCT GGAACTGGAG TAAGGAGTGC CAAAATCCTG GATGAAAGCT GAGCCTGTAC CCCAATGGAG TATAAACGCC GGGGTTTATC AGGGTGTAGG GGGATACGTT GGCTCCAAAC TTCAACTGGC GGAGTAAAGG AACATCAAGA TACAGGAAAG GAGTATTACA ATAAGCAAGA ATCCTAAAGC ACGAAGAAGA GCCCAGCTTT CTTCACGAGT CTGAGCAAGT ATAGTCAGGA AAGCTCCCAG GGGGCAAAAC GAAAAGTTCG GCAGAGAGCG GCAAACAGCT TCTAAAGACC CCCAGCCTGC GAATACGATG CCCTCGATAA AAGGAGCCGG AAGCCGGATG AACTTGGTGG AGAAG TAA TGAGGCCTGG ACAAGGCTAT ACGAAATACC AAGACCTCAG GGGCTATCCT AGTATGCGTT TAGAAAATCA ATGGCAACGT GGGACTGGAG CAAGGTTAGT GGCTGTTGGA CCGAGGAGAT GGTTCAAAGT TTACGGCCTG ATTATGGGTA CAATCGAGAA TCAAGAGGTT TCGCCCCAGA TAATCACCCA AGAAAGGGCT AGAGGGAGTA AGTGGTACCT GGTTTTACCT CATGGTGATA AGGTCCGCAC GATAGATGAG GCTTGAGTAC AAAGAAGAAA CTTATACATA GGGGAGGGAA GAAGATGCTI ATTGGATGAA TAACGTCTCA CCTCAGGAAG CGGCCAGCCC GTGGCAGAAG TGCAGAGGAG TCCAGAAAAG TGAGGAGGCA CGAAATAGCC TTATGGGTAC GGTTGGGCAC CTGGGAGGGG CGAGAGAAGG 2220 2160 2100 2040 1920 1860 1740 1680 1620 1980 1800 1560 1500 1440 1380 1320 1260 1200 1020 1140

JDF-3 - (HMf-like) fusion protein (Fig. 17-R)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

 ${\tt ACCCTCGGGAGGGAGCGAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAA$ ${\tt GCGCTTCTTGAGGGTCGTTAAGGAGAAGGACCCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAAGCGCTGTGAGAAGCTTTGGCGTGAGCTTTT$ ${ t TTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTCGACATGGAGACGCTCTACCACGAGGGAGAAGAGTTTGGAA$ $\mathtt{CAGGTCTGTGGAGGTCCTGTACTTCACGCACCCGCAGGACXXXCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGGGGTCATCGACATCTACGAGTACGACATACCC$ $\mathtt{ACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGGTGAAGAAAAGTTCCTCGG$ $\mathtt{ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCGTCATCAGGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAGCCCTACTTCT$

 $\tt GTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATG$ GTGGCTGAAGCCGAAGGGGAAGAAGAAG// ${\tt CGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGCGC$ ${ t GTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAgcGTTTGGCCGCCAGAGGT$ $\mathtt{ACTGGAGCGAGATAGCGAAGGAAGGCGAAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAA$ ${ t GAACTCGAATACGAGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCCAAGATAACCACGCGCGGGCTTGAGATAGTCAGGCGCG$ $\tt CTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTC$ $\tt ATGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAAAGTCCT$ $\mathtt{CAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCGCCCATCAAGATTCTCGCCAACAGCTACTACGGCTACTACGGCT$ $\mathtt{ACgcCGGTGGCTACGTCAAGGAGCCGGAGCGGGACTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCTTCAATCATAATCACCCACAACGTCTCGCCAGATAC$ GGTCGCGCCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCC

//ATG ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA ACC GIT AAG GIC GAA GAC ATT AAG CIC GCA AIT AAG AGC IGA 162 108

(HMf-like) - JDF-3 fusion protein (Fig. 17-S)

V93R V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

 $// { t ATG}$ ATG GGA GAA TTA CCA ATT GCC CCA GTT GAC AGA CTT ATA AGA AAG GCT GGT GCT CAG AGA GTT AGC GAG CAA GCA GCT AAG GTA CTT GCA GAG CAC CTT GAG GAA AAA GCT ATT GAG ATC GCA AAA AAG GCA GTA GAT CTT GCA AAG CAC GCA GGT AGA ACC GTT AAG GTC GAA GAC ATT AAG CTC GCA ATT AAG AGC // 108 162

 ${ t TCGACATCTACGAGTACGACATACCCTTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTCGACATC$ $\mathtt{GAAGAAAAAGTTCCTCGGCAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACCCGCAGGACXXXCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGCGGTCA$ CCCTACTTCTACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGAGAAGGT GAGACGCTCTACCACGAGGGAGAAGAGTTTGGAACCGGGCCGATTCTGATGATAAGCTACGCCGATGAAAGCGAGGCGCGCGTGATAACCTGGAAGAAGATCGACCT //ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCCGTCATCAGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAG rccttacgttgaggttgtctccaccgagaaggagatgattaagcgcttcttgagggtcgttaaggagaaggacccggacgtgctgataacatacaacggcgacaact

Fig 175 (con+)

GCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGCGCGTGGCTGA GGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATGCGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCC TTGTCAGGGAAGTCACCGAAAAGCTGAGCAAGTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACGG CGGGCTTGAGATAGTCAGGCGCGACTGGAGCGAGATAGCGAAGGAGACGCCAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCGTCAGAA $\tt CTGCCCGGCCTTCTCGAACTCGAATACGAGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCAAGATAACCACGCG$ AAGTCCTCTATGCAGACACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAA TGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTA $\mathtt{AAGAGGAAGATGAAGGCAACTCTCGACCCGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCGCCATCAAGATTCTCGCCAACAGCTACTACGGCTACTACGGCTA$ GTAGGAGCTACGACGTTGCCCCCGAGGTCGGTCACAAGTTCTGCAAGGACTTCCCCGGCTTCATTCCGAGCCTGCTCGGAAACCTGCTGGAGGAAAGGCAGAAGATA GGAGCGGGGACTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCCTTCAATCATAATCACCCACAACGTCTCGCCAGATACGCTCAACCGCGAGGGGGT $\mathtt{AGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCCCGCTCCAGCACCGGCAACCTCGTCGAGTGGTTC$ GCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGCTTGAGAGGGTCGCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACG GTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAAACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAA

ACCESSION No: AE010210 REGION: complement (8333..9082) (Fig. 17-T) /product="pcna sliding clamp (proliferating-cell nuclear antigen)"

ATG S GTT AGA CIC SAGC ACC ATGCTT E GAA GAA ۲ К A S K GCA AGT AAG D GAC s AGC ATG CIT ACA AAG AAA GGA GAG GAA AAC CCA TTT GAA ATC TTTЪ I ATA TTT H L K
CAC CTA AAG R AGG D E G GAT GAG GGA D GAT CCA I ATA < Þ F S AGA A GCC M ATG GCC Q Ħ ഗ GIT M ATG TCT ACT TTA K AAG ۲ Ħ K I BAAG ATC K Y BAA TAT GCA CCC D GAT I ATA V F E G A K E F A Q L I D GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC TTA CTA A GCA Þ Þ ۲ GTG AAG GAT GAG P CCA TIG E GAG \asymp < ۲ U F L E I T I Q G T A T TTC TTA GAG ATA ACA ATT CAA GGA ACT GCA ACA E GAA GTT I D V E E M E ATA GAT GTA GAA GAG ATG GAA CTA S AGT G GGA s AGT D GAC < [X] U I E V : ATC GAG GTT GAC A A F K V
GCC GCG TTT AAA GTT GTA GTT K R G K A K AAG AGA GGT AAA GCA AAG GTT V E GAA R AGA < ⋈ GTT S AGC QTT V T ACC ٧ < < GTC CTT GGA E GAA Q CAG I ATA < Ľ × CIG CAA AAA PCCA E GAA Q × × Q . GAA I E GAG TŢŢ E GAA V GTT < ۲ GTC T ACA GAC ACA E GAG GAG I < G D T GAC ACC GIT A GCC LCTA I ATT CTA GAA T ACA ATA < Ħ D L P GAC CTC CCA D G I A GAT GGG ATA N L AAT CTC AAG AAG AGG K Aaa G GGA GAA AAT D A GAT GCT TTA GIT S AGC CTA < a 9999 ; I ATA N AAC A GCA ACT Ħ 36 108 198 594 162 486 432 144 126 378 108 324 90 270 72 216 54 162 216 180 540 18 54

GIT TAT GGA GTC E GAA ACA GGA R AGA K AAG AGC TAT CTC TCC GAC ATG GTT AAA GGA CTT GGA AAG GCC CTT TTT G N E GGA AAT GAA T ACA TTC CTA M ATG CIG A JJJ 4 PCCA M ATG R AGA CAA GTT ATG GAG E GAA Y TAT Y TAC I GAT R AGA GAA 234 702 250 750 648

(PCNA)-Taq DNA polymerase fusion protein (Fig. 17-U)

ATG ACC T CIC M ATG S S GTT R AGA GAA A GCA CCA P D GAC s AGC M ATG CIT S AGT TTT R AGG H I ATA TTT L K CTA AAG A GCC AAG G GGA TTT GAA ATC GTA R AGA . GAG GTT S AGC M ATG TTA S ACT , AAG I ATA GCA LCTA ეეე ტ E GAA AAA D GAT GIG I PCCA K AAG Y TAT D GAT F E G A K E F A Q
TTT GAA GGT GCA AAA GAG TTT GCC CAA CTA N AAC E GAG S AGT AGT V I ATA TTC LCTA e Gaa A A GCC GCG D V GAT GTA GAC V GTA L E I T I TTA GAG ATA ACA ATT K AAG V GTT R AGA -GTT S AGC VGTT R AGA V GTT GGT E GAA I ATA CTT gaa QTC TTT K A AAA GCA G GGA E GAG P CCA CIG K AAA AAA . GTT E GAA M TTT E GAA I . AAG GTC E GAA CAA T E D ACA GAA GAT D GAC ATT T ACA G T GGA ACT GCC GIT D GAC CTA I LCTA GAC ACC R AGG G GGA N AAT K AAA G I GGG ATA E A GCA I D ATA GAC TTA CIC DGAT CIC GTT V . AAT T ACA IATA PDD P PCCA N AAC 90 270 72 216 162 486 144 432 126 378 108 324 54 162 36 5 1 8

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Y G V S
GAA
                    TACA
                                                              E D E G L L D I E V Q E E T K S A GAA GAT GAG GGA TTA TTG GAC ATC GAG GTT CAA GAG GAG ACA AAG AGC GCA
                                                                                   F I M K A E G E T Q E V E I K L T TTT ATA ATG AAG GCA GAA GCC CAG GAA GTT GAG ATA AAG CTA ACT
G R
GGA"AGA
                     I
ATA
                     .
AAG
CIT
                                          Y
                     TTT
                                         L S D M V K G CTC TCC GAC ATG GTT AAA GGA
T F L L A P R ACA TTC CTA CTG GCT CCA AGA
                    G
GGA
                    N E
AAT GAA
                     M
                     CCC
P
                     ATG
                                                                                                  170 (cont)
                    CAA
QTT
                     M
ATG
                                          CIT
                                          G K
GGA AAG
E E
GAA GAG
                     E
GAG
                     Y
TAT
                     Y I
TAC ATT
                                          A D
GCC GAT
                      R
' AGA
                                          GAA
                      234
702
                                          216
648
                                                                198
594
                                                                                     180
540
        250
```

E GAA

CIT

// GGC GGC GGT

D GAT

GIT

D G H
GAC GGC CAC S A GCC S AGC V T S G $^{\rm M}$ L P L F E P K G R V L L V GTC ACT AGT GGG ATG CTG CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG GTG TTC CGG CIC TTTAAG CAC CCC H E A Y G G CAC GAG GCC TAC GGG GGG CGG R P CCG L A Y R T F H A CTG GCC TAC CGC ACC TTC CAC GCC D GAC CAA OTG V 999 9 L A D A GAC GCG Q A X Y CAG GCG OTC TAC CIC V GTG ATC Y TAC I ATC K A AAG GCG AAG GIG 999 9 GAG TTC QTC L K G A GCC CIG 9 9 9 TTT R A CGG GCC K S AAG AGC D A GAC GCC V GTG GAC L T T CIC CIC CIC) J K AAG CIG T ACG AAG CCA P ეეე ქ

A GCC GCC A CIG AGG CIG 9 9 A GCC GAC acc A TTC CIC CTG CIG V GTG A GCC ეეე ტ HCAC CiC ATC DGAC I ATC CIT A GCG GAA K K A E K AAG AAG GCG GAA AAG A A T P A Y Q L TAC CAG CTC TTT CIG M D D L ATG GAC GAT CTG L K CTC AAG Y TAC CGC S AGC **G**GG E GAG DDD P CTG GAG CGG CIC V GTG N AAC R AGG K AAG E GAG A GCC E GAG V GTG K AAG မှ မှ CII A GCC R AGG GAC CIG T ACG CTG TGG L S D R I H V L H
CTT TCC GAC CGC ATC CAC GTC CTC CAC QTC CIT FTTC K AAG D GAC T ACC CIT E GAG ရှိ ရ CIG A GCG TCC වටට අ FIG IN (CONT)

G Y E A D D V

G GGC TAC GAG GCG GAC GTC C E GAG A GCC CIC **G**GG TGG GGC TAC GAG GTC CGC ATC CGG CGC R E GAG CGG R AGG K R C AAA AGG F G TTT GGC D E GAC GAG OTC E GAG S W D L
TCC TGG GAC CTG E K Y G GAA AAG TAC GGC AAG CIG AAG : CAC GCC AAG CIT s AGC CGG R 233 P CGG R 222 A TCC ეეე ჭ CIG CIC E GAG A GCC A GCC D GAC W TGG E GAG GCC CIG CIC DDD P I ATC ეეეე გ N AAC TGG 222 4 E GAG E GAG CAC D GAC K AAG CIT R AGG CIC GCC A 933 4 CGG R TGG GTG P P E GAG CGG R E GAG P E G T A D ACC GCC GAC CIG CCI D GAT P P 9 9 9 ეეე ჭ R T GC ACC TTC GCC A E GAG AAG GAC D Y TAT CII E GAA 999 999 S AGC ATC Q CAG S AGC AAA K 9 9 9 9 9 R AGG GTC Cic CIG T D GAC GCC A CIG AAG TGG YTAC AAA GCC A CIT CIT GAA GCC

Fig 170 (cont)

96C S A GCC R CGC E GAG A GCC . GGG CIC Y TAC A GCC CIC AAG R D L AGG GAC CTG K AAG R CGG CGC CIG QTG N AAC CTC CTG GAC CCT TCC AAC ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC OIC T E W T E E A G E R A A L S E R L GAG TGG ACG GAG GAG GCG GAG CGG GCC CTT TCC GAG AGG CTC D Q GAC CAG D V A GAC GTG GCC T E K ACG GAG AAG L W G R L E G E E R L CTG TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTC GAG GCC GAG GTC E R P L GAG AGG CCC CTT R E G L G AGG GAA GGC CTT GGC CIG T ACC AAG T G K E R GAA AGG Y TAC ეეე გ Y TAT E GAG ZJ ATC CIC S I A GCG 긔 V GTG V L F D E L G GTC CTC TTT GAC GAG CTA GGG GCT R G CGG GGG D GAC TTC R AGG L P P G D D P M L L CTC CCG CCC GGC GAC GAC CCC ATG CTC CTC A L S 222 P E K I L Q GAG AAG ATC CTG CAG R S T S A A V L E CGC TCC ACC AGC GCC GCC GTC CTG GAG V L A H M GTC CTG GCC CAC ATG R L A G H O TIG L L A K
CTT CTC GCC AAA CCG P D GAC CIG CIC E GAG L W L Y D 4 QTG D L S V GAC CTG AGC GTT I E A T G GAG GCC ACG GGG L P A R E L CGG GAG CTC F N L A E E GCC GAG GAG H R AGG Y AAC T ACG I I ATC V GTG CGG R A GCC ACC acc A TTC

Fig 17 U (cont)

V GTG 9 9 9 CGG AAG I N AAC S AGC GAA ACC ATC S AAC S E GAG ∨ GTG CCT F V GTC GTC Q CGC R SS G V I E ATA GAG GIG F Q Y E TAC GAG T ACC P \swarrow_R E A V D P L M R R CCC CGG GAG GCC GTG GAC CCC CTG ATG CGC CGG CIC CGG R GCC CGG R CIC A GCC A GCC CAC ACC CGC TTC E G GAG GGG E GAG CIC CIC E GAG TTC TGG TTC F AGG ATC GCG A GCC Y TAC R AGG CIC မရှင် ရ I ATC Q CAG 999 9 CGG R V GTG A GCC CAG CIC S S S E GAG A GCC AAG D I H T E T A S W M GAC ATC CAC ACG GAG ACC GCC AGC TGG ATG A GCC M S A H R ATG TCG GCC CAC CGC CTT E GAG CIG AAC CGC R K AAG E GAG N AAC A H GCC CAC F I TTC ATT CAG Q CAG CGC T ACC I CGC R E GAG GIC V M ATG Y CIG G G G 222 4 ACG GCC CIC TCC GCC E GAG H V GTG V GTG OIC A E GAG TGG W D GAC CGC R CGC R F CCA. E GAG LCTA ACG GCC ACG GGC AGG Y N AAC CIC T ACC D GAC 9 9 9 ၁၅၅ ၁၅ TIG S D GAC TTT A A K T I GCG GCC AAG ACC ATC CTG TTC M ATG LCTA R. AGG GTG 933 4 E GAG CAG Q CAG QTC V 222 P gag R AGG A GCC CIT E L GAG CTA GIC N AAC GCC A S AGC CIC R CGG CIG Q CAG CIG GAG CIG R CGG TTC CTA AG1 မှ မှ မှ GCC A 222 4 GCC 999 9 V GTG TAC TTC I AGG GAG Y TAT

P CCA K E R A E A V A R L A K E V AAA GAG AGG GCG GAG GCC GTG GCC CGG CTG GCC AAG GAG GTC M E G C ATG GAG GGG

CIC v GTG S A K E G I D G R G G G H H H H
TCC GCC AAG GAG GGC ATT GAT GGC CGC GGC GGA GGC GGG CAT CAT CAT Y P L A V P L E V E V G I G CTG GAG GTG GGG ATA GGG E D W

H H *
CAT CAT TAA

aq DNA polymerase- (PCNA) fusion protein (Fig. 17-V)

// GGC GGC GGT

D GAC GTC G H H L A Y R T F H A L K G L T T GGC CAC CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC CTC ACC ACC T ACT S G M L P AGT GGG ATG CTG CCC L F E P K G R V L L V CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG GTG

S R CGG G E P V Q A X Y G F A K S L L K GGG GAG CCG GTG CAG GCG OTC TAC GGC TTC GCC AAG AGC CTC CTC AAG

A GCC Cic K E D G D AAG GAG GAC GGG GAC A GCG V I V V LLL GAC A GCC K A PAG GCC 222 d

S TTC R H E A Y
CGC CAC GAG GCC TAC 9 9 999 9 Y K TAC AAG A GCG 9 9 CGG A GCC 222 P T ACG P CCA

222 d R CGG CAA CIC GCC A L I K E GAG L V D CTG GTG GAC CIC CIG မှ မှ

Fig 17v (cont)

A GCC A GCC 9 9 A A A GCC CIC D GAC CIG TTC CIG R AGG CIG 222 4 CAC I gac I ATC CIT K AAG A GCC CIC A GCG M D D ATG GAC GAT T P A Y Q CIG G G G G Y TAC K A E K AAG GCG GAA AAG CIC CGC R R A Gege GCC E V GAG GTG CIC E GAG ეეე ტ CIG AAG CIC K AAG K AAG E GAG V GTG E GAG AAC W L CIT A GCC D GAC CIG T ACG CIG CTT QTC R AGG CIG F K AAG A GCG T ACC S E GAG CIG CII D GAC P P S CIC 999 9 မှ မှ A GCC w TGG D R I H V GAC CGC ATC CAC GTC E GAG E GAG CGG R AGG 9 9 9 CGC R K R R C AAA AGG CGG D E S GAC GAG TCC Y E V F G S Y E A E K Y G GAA AAG TAC GGC S AAG E GAG K AAG CIG W A GCC CIT E GAG K AAG D D D GAC CCC CIG , p R I CGC ATC E GAG CIG D GAC D GAC M ATG W TGG CIC A GCC E GAG L H A GCC N AAC 555 Å CIG 222 A E GAG TGG CIC I D GAC שי D GAC CIT R AGG CIC AAG W TGG GTC A GCC 933 4 CAC CGG CGG P E P D V GTG T ACC CGG 9 9 9 333 d D GAT 500 A E GAG E GAG CIG R T CGC ACC gcc A GCC E GAG 999 999 CII E GAA TTC K AAG S к D GAC R AGG V GTC Ç CAG 999 9 S AGC GGG 999 9 I CIG AAA K GCC A A GCC Cir CTT GAC CIG GAA AAG TGG Y CIG

F917 (cont)

CGC R A GCC 9 999 Y A GCC 9 9 S a GCC E GAG CIC CIG AAG QTG N AAC CIC CIG E W T E E A G E R A A L S E R L GAG TGG ACG GAG GCG GGG GAG CGG GCC GCC CTT TCC GAG AGG CTC K AAG cgg R CGC R CIG R AGG E R P GAG AGG CCC D Q GAC CAG K S T L :5W G R L E G E E R L L W L Y R CTG TGG GGG AGG CTT GAG GGG GAG GAG AGG CTC CTT TGG CTT TAC CGG R E AGG GAA T E K T G ACG GAG AAG ACC GGC CIC D V A GAC GTG GCC L D P S N T CTG GAC CCT TCC AAC ACC GCC GCC AGG GGG GGC CGG GTC CAC CGG GCC CCC GAG CCT TAT AAA GCC E GAG CTG A GCC Y L TAT CTC Y I TAC ATT CIT E GAA E GAG CIT TCC R AGG GIC A GCG K AAG GIC R AGG A V L A H M E A T GCT GTC CTG GCC CAC ATG GAG GCC ACG CIC CGG R TIC A GCC T ACC R CGC CIC CGC R 900 4 G G G K I AAG ATC TIG TCC S T S A A T S A C TCC ACC AGC GCC GCC F D TTT GAC P E G V CCC GAG GGG GTG CIT TTG CIG 555 P DDD P A . 299 9 CIC L Q CTG CAG D GAC E GAG CIG GAC A GCC 9 9 9 CIC LCTA CAC E GAG D GAC AAA V A GTG GCC A GCC TAC . GAC ATC 9 9 DDD 4 222 4 GTC V CGG R CIG CGG CTT ATG TTC P A CGC GAG CIG gag CIC DDD P AAC G V E I GAG ATC Y AGG CIC E GAG CIC F ှိ ရှိ ရ A GCC I ATC CIG T ACG T ACC AAC

Fig 17V (cont)

T ACC AAG OIG V AAG ATC N AAC 9 9 R CGG S I 999 9 S F G V R R A R L CGC CTC E GAG OTG A LDD Å GTC V GTC Q CAG S D P N L
TCC GAT CCC AAC CTC R A CGG GCC ය අ Y E E A Q
TAC GAG GAG GCC CAG T ACC 222 Å I ATA TTC GCC CTC CGG GAG H T Q CAG CIC R AGG TGG CIC TTC E GAG E GAG CIC TTC M ATG 9 9 9 I TAC A GCC ATC R AGG CGC R 999 9 E GAG 999 9 CIC CGC R ole V Q N I P V R T CAG AAC ATC CCC GTC CGC ACC TTC AAG V GTG A GCC CGG R E E G K T L E E AAG ACC CTG GAG GAG A F I E R Y GCC TTC ATT GAG CGC TAC M ATG CIT CIG L E GAG CGC R D GAC D GAC CIG N Q T A T A T G R AAC CAG ACG GCC ACG GGC AGG R Y V CGC TAC GTG A H GCC CAC 333 4 CAG AGCT S A H R TCG GCC CAC CGC 290 R I ATC GTC M ATG CIG H CAC TGG V M ATG CIC A GCC T ACG GAC P TCC AAG TTC CGC R E GAG CTA CIC E GAG D GAC 9 9 9 CGG CHC L T ACC 9 9 9 TIG N AAC R AGG TTT TCC 999 4 LCTA A GCG GCC A GAC GIG TIC ATG A GCC GTC R AGG Q CAG CAG .GCC CTT ეეე ტ SSS A E GAG S AGC E GAG K T BAG ACC E L GAG CTA G Q GGG CAG S AGC CGG R CIC R AGG A GCC TGG AAC N CIG : GAG 9 9 9 L S CTA AGT TTC CIG CAG CGG R M ATG CIG D GAC GCC A A GCC TAC Y TAT GAG QTG ටටට අ ATC ATC 9 9 9 TTC

V A R L A K E V M E G GTG GCC CGG CTG GCC AAG GAG GTC ATG GAG GGG FIS 17 V (cont)

CCA

AAA AAA

E R A GAG AGG GCG

E A GAG GCC

CIC V GTG S Y TAT A K E G I D G R G GCC AAG GAG GGC ATT GAT GGC CGC GGC D D CIG A GCC V P L E V E CTG GAG GTG GAG G GGA ole A GGC GGG CAT CAT CAT 9 9 9 I ATA G E E D GAC TGG

z AGC T ACC P ATG CAT STCA M A S K L I D E A A F K V T E D G I GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA F E \downarrow V F E G A K E F A Q L I D CCA TIT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC S CAT I ATA R A M D P S R \dot{V} V AGG GCC ATG GAT CCA AGT AGA GTT GTC :: F S K Y E V AAA TAT GAA GTT GTT V GAA L I CTG ATT CCA P gaa GAC T ACA I ATT LCTA N L P AAT CTC CCG GGA A GTT N AAC 72 216 54 162 36 108

54

AAG CTT K AAA CCA TTC R V AGA GTT G E E N F L E I T I Q GGA GAG GAA AAC TTC TTA GAG ATA ACA ATT CAA T ACT A K V V V L
GCA AAG GTT GTA GTT CTT P L I D V E E M E CCC CTA ATA GAT GTA GAA GAG ATG GAA G E V GGA GAA GTC G T GGA ACT . GTT L K D A CTA AAA GAT GCT GAC A T GCA ACA CIC CCA P 144 432 126 378 108 324

AGA

M ATG

D GAC

CAC

L K CTA AAG

K AAG

ATC

CTA

K R G A AAG AGA GGT

K A K D T L I AAA GCA AAG GAC ACC TTA ATA

90 270

CIC

GAT GAA YAT CII GTT < G GGA AAA V S G R L T GGA AGA CTT ACA D E G GAT GAG GGA D A S L GAT GCC TCT CTA aag ATG Y TAT TTT AAG CIC L TTA GCA GGA TIC N AAT GAG TCC V S D S I K
GTG AGT GAC AGC ATA AAA TIG LCTA -GAA D GAC D GAC G E T Q E GGA GAA ACC CAG GAA ATG L A P R V E E CTG GCT CCA AGA GTT GAA GAG M ATG I E V Q ATC GAG GTT CAA 222 A QTT M ATG K Aaa G G A CAA M ATG GAG CIT GIT TTTGAG E GAG E GAG ATT GCC Y Y I TAT TAC ATT I K HATA AAG T ACA . AAG TGA gcc A AGG GAT L CTA GAA S AGC R AGA GAA GCA ACT T AAT 250 234 702 216 648 198 594 540 162 486 180

Pfu DNA Polymerase (WT) - (PCNA) fusion protein (Fig. 17-W)

ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc tctaacctgg gttatagtga caaatcttcc tccaccacg cccaagaagg ttatttctat caactctaca cctcccctat tttctcttt atgagatttt taagtatagt tatagagaag gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga ttacataact gaagaaggaa aacctgttat taggctattc aaaaaaagaga acggaaaatt taagatagag catgataga cttttagacc atacatttac gctcttctca gggatgattc aaaagattga ggaaggttgaga aaaataacggg ggaaaggcat ggaaagattg tgagaacttta tgatgtagaa aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta

TO TW (CONT)

ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc tagageceta tatecetega ttataattae eeacaatgtt tetecegata etetaaatet aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag aataccaatg gagggggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct

FIG ITW (cont)

aggogatggt ccaattagca atagggcaat totagotgag gaatacgato ccaaaaagca gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac gaaggagctc gaagaaaagt ttggatttaa agtcctctac attgacactg atggtctcta gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatggt aacttcctgg cttaacatta aaaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag atacataaat tcaaagctcc ctggactgct agagcttgaa tatgaagggt tttataagag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta

M3.17W (cont)

gtagaagcgg cactactcag atgcttcccc aggaatgagg ttgttgtagc tcntcccnga gacactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg gttgagaaaa ctcgatttcc ttggttgtgc tccatatgat aagcttccaa agtgggtgtt cagactttta cagagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct ccagggtaat gtttttaagt atgaaatttt tctttcatag aggaggnnnn nngtcctctc aaagtataat ttcaagctcc ttcttcttca tgacagatga aaaattgttt tgtctctttt aagattgaga tgttcttgg taacttttac agaaataact gtctcaaatt atgacaactc ttgacatttt tacttcatta ttccgggagt tttgtattgc tctagccaag gtttgggatt tttgaatcct ttaactctgg acaatttttt ccttgtatct cctaatgtat aagcaagcca aaggagagta gatgctacct tttgctccaa gcagagccgc tccaatggat aacacccctg ttcccgcacc caagtccgct tcaagatttt ctaaaagaat tttaacggcc tcctcgtcaa tttcgacgac gtagatcttt ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct

TCA ATG CTC GAA AGA AGC AGC GAC AAG ACA ATG ATA ATG AAG GCA GAG GAT GAG GGA TTA TTG CCA TTT AAA CAC ATA AGG AGT GAT GCC TIC AGA CTA GGA GCC ATG GAA AAG TTT AGC AAA TAT A AAG AAG ATC A GAG GAA AAC TTA GCA AAG GAT CCA GAA ATA AGT CTA GTA TTA AAG AGA GCC ACC GAG AGC GIT GTA GAG AGA GTT GTT GCG GCA ATA GAA ATA GAA GIC GGT AAA GGA ACA CCA AAA AAA TTT AAA GCA . GAA ATG ATT GAA ACA GAA CAA AAG ACA GAC GGA GAC AGG AAA GAC ACT ACC GGA AAT GAT GAT TTA ATA
GCA ACA
CTC CCA GAA GTT CIC 999 GCI AAC ATA ATA AAT 432 486 540 594 54 108 162 216 270 270 324 378

,

TAT GGA GTC AGC TAT CTC TCC GAC ATG GTT AAA GGA CTT GGA AAG GCC GAT GAA GTT ACA ATA AAG TTT GGA AAT GAA ATG CCC ATG CAA ATG GAG TAT TAC ATT AGA GAT GAA GGA AGA CTT ACA TTC CTA CTG GCT CCA AGA GTT GAA GAG TGA

648 702

(PCNA) - Pfu DNA Polymerase (WT) fusion protein (Fig. 17-X)

GAT	GTT	TAT	CTT	GAA	GTT	GAA	AGA	CIC	ATG	TCA	AGC	ACC	ATG
GAA	ACA	GGA	GAA	TTT	AAA	CIT	ACA	AAG	GAC	AGC	ATG	GCA	CCA
GGA	ATA	GIC	GAT	ATA	GAT	CCA	TTT	AAA	CAC	ATA	AGG	AGT	TTT
AGA	AAG	AGC	GAG	ATG	355	TIC	AGA	GGA	CTA	TTT	GCC	AAG	GAA
CIT	TTT	TAT	GGA	AAG	TCT	ACT	GTT	GAG	AAG	AGC	ATG	TTA	ATC
ACA	GGA	CTC	TTA	GCA	CTA	GCA	CCC	GAA	AAG	AAA	GAT	ATA	GTA
TTC	AAT	TCC	TTG	GAG	GTG	AAG	CTA	AAC	ATC	TAT	CCA	GAT	TTT
CTA	GAA	GAC	GAC	GGA	AGT	GTT	ATA	TTC	CTA	GAA	AGT	GAG	GAA
CIG	ATG	ATG	ATC	GAA	GAC	GTA	GAT	TTA	AAG	GIT	AGA	GCC	GGT
GCT	CCC	GTT	GAG	ACC	AGC	GTT	GTA	GAG	AGA	GIT	GTT	GCG	GCA
CCA	ATG	AAA	GTT	CAG	ATA	CTT	GAA	ATA	GGT	GAA	GTC	TTT	AAA
AGA	CAA	GGA	CAA	GAA	AAA	GGA	GAG	ACA	AAA	CCA	CTG	AAA	GAG
GIT	ATG	CTT	GAG	GTT	TTT	GAA	ATG	ATT	GCA	GAA	ATT	GTT	TTT
GAA	GAG	GGA	GAG	GAG	ATT	GIC	GAA	CAA	AAG	ACA	GAC	ACA	GCC
GAG	TAT	AAG	ACA	ATA	ദ്രാ	CTA	GTT	GGA	GAC	ATT	CTA	GAA	CAA
	TAC	GCC	AAG	AAG	AGG	AAA	GAC	ACT	ACC	GGA	AAT	GAT	CTT
	ATT	GAT	AGC	CTA	GAA	GAT	CIC	GCA	TTA	GTT	CIC	GGG	ATA
	AGA	GAA	GCA	ACT	AAT	GCT	CCA	ACA	ATA	AAC	CCG	ATA	GAC
	702	648	594	540	486	432	378	324	270	216	162	108	54

ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat caactctaca cctcccctat tttctcttt atgagatttt taagtatagt tatagagaag gttttatact ccaaactgag ttagtagata tgtgggagc ataatgattt tagatgtgga ttacataact gaagaaggaa aacctgttat taggctattc aaaaaagaga acggaaaatt taagatgaa ggaaaggcat ggaaaggttca ggaaagttga gaagttaaga aaataacggg ggaaaggcat ggaaagattg tgagaattgt

 \approx

Tig 17.x (cont)

gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga tagagcccta tatccctcga ttataattac ccacaatgtt tctcccgata ctctaaatct ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa aataccaatg gagggggaag aagagctaaa gattettgee ttegatatag aaaceeteta tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tgatgtagag aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta

Fg 17x (cont)

gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta aacttcctgg cttaacatta aaaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt aggcgatggt ccaattagca atagggcaat tctagctgag gaatacgatc ccaaaaagca actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag gggattette gttaégaaga agaggtatge agtaatagat gaagaaggaa aagteattae atacataaat tcaaagctcc ctggactgct agagcttgaa tatgaagggt tttataagag gaaggagctc gaagaaaagt ttggatttaa agtcctctac attgacactg atggtctcta ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa

tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatgt ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct caggagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct tcaaagatttt ctaaaaagaat tttaaccgcc tcctcgtcaa tttcgaacgac gtagatcttt tttgctccaa gcaggacgc tccaaatggat aacacccctg ttccggacg cgaggatcttt ttccggggagt tttgtattgc tctaagcaag gtttgggatt tttgaatcct ttccgggagt tttcgggagt ttttgaatct ttcaagccaag gtttgggatt tttgaatcct ttaactctgg aaagttttac agaaataact gtcttcttca tgacaagcca aaggaggagtatttt tgtctctttt tacttttac agaaattact ttctaagccaaatt tttttcatag aggaggnnnn nngtcctctc ctcgatttcc ttggttgtgc tccatatgat aagcttccaa agtgggtgtt cagactttta gacaactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg ttgttgaaaaa gtagaagcgg cactactcag atgcttcccc aggaatgag ttgttgtagc tcntcccnga aagattgaga tgttcttgg // TGA

(PCNA) - PFU DNA POLYMERASE (V93 R OR E) fusion protein (Fig. 17-Y)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)
V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA 54 108

F917Y (cont)

GIT ATG GAA AGA TAT GAA CIC ACA GAC GAA AAA AAG AGC ATG TTT ATA ATG AAA CAC ATA AGG GAT GCC CCA TTT AGA GAG AAG AGC GGA GCC TIC TAT GGA AAG ACT GTT GAG AAG ATG CCC GCA GAA AAA TTA GCA AAG TTC AAT GTG TAT TCC TIG GAG AAG CTA AAC ATC GAA GAC GAC GGA ATA GIT TTC CTA AGT GAC AGC ATG CCC ATG ATC GAG GAT GAA ACC GTA CIG GCI GTA GTT GAG GIT AGA ATG AAA GGT GAA CAG ATA GAA ATA CAA CAA GGA GAG ACA AGA GAA AAA GGA AAA CCA TTT ATT GCA GAA GIT ATG GAG GTT GAA ATG CTT GGA GAG ATT GIC GAA AAG GAC ACC GAA CAA GAG TAT TAC GAG ATA AAG ACA ATT GGA GAG // ACA GTT GAC GGA ACT AAG GCC AGG CTA GCC AAA GCA GAT AGC CTA GAA GAT CTC TTA ATT AGA ATA AAT GAA GCA ACT GCI CCA ACA AAC 594 486 432 378 540 324 270

TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GCAAAAGCAA GAIGGIACIG TAAGGAGIGI GCIGAGAGCG TIACIGCCIG GGGAAGAAAG 1560 IACAICGAGI TAGIAIGGAA GGAGCICGAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900 ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GAGTTTGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC 360 AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 1740 GAAGGGTTTT ATAAGAGGGG 720 540

2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA

PFU DNA POLYMERASE (V93 R OR E) - (PCNA) fusion protein (Fig. 17-Z)

GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAAT AAAGAAAAAG GTTGAGGTTG GCAAAAGCAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 AGGACATCCC CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA GGAGACTCAT ATGATTTTAG ATGTGGATTA CATAACTGAA GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 ССТСТАБААТ ТТБТААААТА САТАААТТСА ААССТСССТБ GACTGCTAGA GCTTGAATAT TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA ATGCAGAGAA GAGTTTGGAA CTCATCGACA CAAGATXXXC CCACTATTAG AGAAAAAGTT AAGATTGTGA GAATTGTTGA TGTAGAGAAG GATAGAACTT TTAGACCATA CATTTACGCT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA CACTAGAGGC TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TCGCATTCCC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG AAGGCCCAAT TATAATGATT AAGGCCTAAT ACCAATGGAG TATCAAGCGA GAGAGAGATG TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCC 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 TATCATGTAA TAACAAGGAC AAGGATCCTG ACATTATAGT ATTACTTGGA AAAACATAGA GATATAGAAA CCCTCTATCA TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TACATCGAGT TAGTATGGAA GGAGCTCGAA TTAAGACAAA AATGAAGGAA ACTCAAGATC 1740 GAAGGGTTTT ATAAGAGGGG AATAAATCTC TCTTCCATAC CGAAGGAGAA TACTTATAAT 360 540 720

ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG TCA ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA AGC ATG ATG AGC GAC ACA AAG AAA GAT AAA ATA AGG GCC ATG CAC CTA AAG CCA GGA GAG AAG G AGC AGA TTC TTT AGC ACT GIT GAA GAT AAC CCA AGT AGA GTT GTC CTG ATT GAG AAG AGT GAC GGA ATA JIC GAA GIT GIT AAG GAA GAC GAT GTA TTA ACC GAG AGC AGA GAA CCA GGT AAA AAA GAG GGA GAA GAA GAC AAG ACA GAG GAG GAG ATT AAG ACA ATA GCC GTT GGA GAC CTA AAT CTC GGA ACC TGA GCC AAG AAG ACT AGG GTT AAC GCA ACA AGC TTA ATA CTA GAA GAT : cce E G t CC 5<u>4</u> 648 486 432 378 270 216 162 108 594 540

PFU DNA POLYMERASE (G387P/V93R OR E)-(PCNA) fusion protein (Fig. 17-AA)

AGTGGAGAGA CCAACATACA GGAGACTCAT GTTGAGGTTG GAGTTTGGAA CAAGATXXXC AAGATTGTGA GAATTGTTGA GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T) CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA TCGCATTCCC ACCTTGAGAG CACTAGAGGC TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA AAGGCCCAAT TATAATGATT CCACTATTAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 AGAAAAAGTT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC TACGCCGACG AGATAGCAAA AGCCTGGGAA ATTACTTGGA AAAACATAGA TCTTCCATAC ACCGTGTGGA AACTTTATTT GGAACATCCC TAACAAGGAC AATAAATCTC 900 720 540 360 180

NGGATTCGTT AAAGAGCCAG AAAAGGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA 2280 ACAAGACAAG: TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC:// GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAA GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACACC TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG 1680 GCTCTAGAAT TIGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG TTAAGACAAA AATGAAGGAA ACTCAAGATC 2328 AAAAGCACAA GTATGACGCA

ATG TAT GTT GAA GAA AGA TCA AGC ATA TTT AGC AGC ATG AGG GCC ATG ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA CIT $^\prime/$ ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC ACA GAC CAC CTA AAG AAA GAA AAG AAA GIC GAT CCA ATA ATG AAG GAT GCC TTT AGA GTT GAG AGC TTC ACT GGA GAG GGA TCT CCC AAG AAA GAT CCA AGT AGA GTT GTC GCA CTA GCA GAA ATC TAT TTC TCC TIG GAG GIG AAG AAC ATA CTA CTA GAA GAC GAC GGA AGT GTT TTC GAA GTT GTT GAA ATG AAG AGA GGT AAA ATG ATC GAA GAC GTA GAT GTA TTA CCC ATG GAG GTT ACC CAG AGC ATA GTT GAG ATA AAA GAA GGA CCA CAA GAA AAA GAG ACA CTG ATT GCA GAA GAG GAA ATG ATT . ACA GAC AAG ATT GAA GAA GAG GGA GAG GTC CAA GAG GAG AAG GCC GTT GGA GAC ACC TTA ATA ATT GGA GTT AAC CTA AAT CTC ACA ATA TAT TAC CTA GAC 335 AAG ACT GCA ACA AAG AAA AGG GAA AAT CIC AGC GAT GCT CTA GAT GAA GCA CCA CCG ACT 270 108 594 486 432 378 324 216 162 648 540

(PCNA) - PFU DNA POLYMERASE (G387P/V93R OR E) fusion protein (Fig. 17-BB)

Fig. 17BB (cont)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T)

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ATG CCA TIT GAA ATC GTA TIT GAA GGT GCA AAA GAG TIT GCC CAA CIT ATA GAC
                                                                                                          ATG
                                                                                                                                             ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA
                                                                       GAA
                                                                                                                       TCA
                                                                                                                                 AGC ATG AGG GCC ATG
                                                                                 AGA ACA
                                                                                                                      AGC ATA TTT AGC
            ACA ATA AAG TTT
                      GGA GTC AGC TAT
                                                            AAA
                                                                                              AAG AAA GGA
                                                                                                         GAC CAC CTA AAG
                                                                       CCA
                                   GAT GAG GGA
                                                          GAT GCC
                                                                                  TTT AGA GTT
                                                                      TTC ACT
                                                                                              GAG
                                               AAG
            GGA
                                                                                  CCC
                                                                       GCA
                                                                                                           AAG
                                                                                               GAA
                                                                                                                       AAA
                                                                                                                                  GAT CCA AGT AGA GTT GTC CTG
TTC
                                                                                                          ATC
                                                          GIG
                                                                                               AAC
                                                                                                                      TAT GAA GTT GTT GAA
           AAT GAA ATG CCC ATG CAA ATG
                        TCC
                                   TTG
                                               GAG
                                                                       AAG
                                                                                  ATA
                                                                                              TTC
                                               GGA
                                                          AGT
CTA CTG GCT CCA AGA GTT
                       GAC ATG GTT AAA
                                   GAC ATC GAG GTT
                                                                      GTT
                                                                                                          CTA AAG AGA GGT
                                                                                 GAT GTA GAA
                                               GAA ACC CAG
                                                          GAC AGC ATA
                                                                                              TTA GAG ATA
                                                                       GIT CIT
                       GGA
                                                                      GGA
                                                                                  GAG
                                    CAA
                                                          AAA
                                                                                               ACA
                                                                                                                      CCA
                                                                                                           AAA GCA
                                                                                  ATG
                                                                                               ATT
                                                                                                                      GAA
                                    GAG
                                                           LTT
                                                                       GAA
                                                                                                                                  ATT GAC CTA AAT CTC CCG
                                                                                  GAA
                                                                                                          AAG
GAA GAG
            GAG TAT TAC ATT AGA
                       GGA AAG GCC
                                  GAG
                                              GAG
                                                          ATT
                                                                      GTC
                                                                                              CAA GGA
                                                                                                                      ACA ATT GGA GTT AAC
                                    ACA
                                              ATA
                                                          900
                                                                                 GTT GAC CTC CCA
                                                                                                          GAC ACC TTA ATA
                                                                       CTA
                                                                                              ACT GCA ACA
                                    AAG
                                               AAG
                                                          AGG GAA AAT
                                                                      AAA GAT GCT
                                              CTA ACI
                                  AGC GCA
                                                                                 378
                                                                                              324
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GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACACC ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GAGTTTGGAA CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC NGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG CGAAGGAGAA AAAGAGATAC 360 720

GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC //TAG GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG GGAGGAGAA GTGAGGAAAT AAAGAAAAAG AAAAGCACAA GTATGACGCA

(PCNA)-PFU DNA POLYMERASE (D141A/E143A/V93R OR E) fusion protein (Fig. 17-CC)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T)

ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC CTT GAA GIT AGA ATG AGC ATG AGG GCC ATG ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA TCA AGC ATA TTT AGC GAA AAG GAC AAA ACA GAA GAT GAG TTTGGA GTC AGC AAA GAT GCC CAC CTA AAG ATA ATG CCA TTT AGA GGA GAG GIT TCT AAG GCA GCA CCC GAA AAG AAA CCA AGT AGA GTT GTC AAT TTG GAG GTG TAT TCC AAG AAC GAA GAC ATA GAA AGT GIT GAC ATG GTT AAA TTC GTT GTT GAA ATC GAG GTT GAT GTA GAA AAG AGA GGT ATG CCC ATG GAA ACC CAG GTA GAC AGC TTA GAG ATA GIT ATA CAA GAA AAA GGA GAG AAA CCA GAG GIT GAA ATG GAA . ACA GAC CTA AAT CTC CCG GAG GAG ATT CAA AAG GGA GAG GIC GAA GTT GGA ATT ACA ATA GCC GAC ACC TAT TAC ATT AGA AAG GCC CTA AAG AAG AGG GAC CTC CCI ACT GGA GTT AAC AAA GAT GCT CTA ACI GAA AA1 TTA ATF GAT GAA AGC GCA GCA ACF 162 108 594 540 486 432 378 324

GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 ATAAGAGGGG AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT GCTACACAGG.TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA ATGCAGAGAA GGAGACTCAT GTTGAGGTTG GAGTTTGGAA CTCATCGACA CAAGATXXXC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TCGCATTCCC AAGGCCCAAT TATAATGATT AAGGCCTAAT TATCAAGCGA CCACTATTAG ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG GAGAGAGATG ACCAATGGAG AGAAAAAGTT ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATC GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 AAGGATCCTG ACATTATAGT ATTACTTGGA AAAACATAGA GAATACGATA TTCCATTTGC TATCATGTAA TAACAAGGAC GCNATAGCNA CCCTCTATCA CTCGCAATAT ATGAGCAGAT AACAAGACCA TCATTACTCG TGGTTTAGAG ATAGTTAGGA GCTTGAATAT 1740 GAAGGGTTTT AACTATCCCA GGAGGAGAAA GTGAGGAAAT 1560 TACATCGAGT TAGTATGGAA AATAAATCTC CGAGCCCAAG CGAAGGAGAA TACTTATAAT TCTTCCATAC AAAGAGATAC 900 360 540 720

TGA

PFU DNA POLYMERASE (D141A/E143A/V93R OR E) - (PCNA) fusion protein (Fig. 17-DD)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T) V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGGA AAGGCATGGA 180 ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT

Fig. 17 DD (cont)

GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA AAAGAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 GCTACACAGG.TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CTCATCGACA CHAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA ATGCAGAGAA TAGGCGATAT GTTGAGGTTG GAGTTTGGAA AAGATTGTGA GAATTGTTGA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC TATCAAGCGA AAGGCCTAAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC GAGAGAGATG ACCAATGGAG TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GCNATAGCNA CCCTCTATCA CTCGCAATAT ATGAGCAGAT AACAAGACCA AACTATCCCA GGAGGAGAAA GTGAGGAAAT 1560 TACATCGAGT TAGTATGGAA 2328 AGCCTGGGAA CGAGCCCAAG TACTTATAAT CGAAGGAGAA AAAGAGATAC AATAAATCTC 720 360

// ATG GTT ACC GAA GAA AGA ATG TCA AGC AGC ATG GCA CCA AAA ACA AAG GAC CTT AGT .AAG ATA AGG GCC TTT GAA ATC CCA AAA CAC TTT GCC AGA GGA CTA TTT AGC GIT AAG TCT GAG AAG ATG TTA ATA GTA CCC GAT GAA AAA AAG TTT GTG CCA GAT GAG ATC TAT TIG AAG AAC AGT GAG GAA GGT GAC GAC GGA AGT GIT ATA TIC CTA GAA SSP AGA GAC GAT GIT ATC GAA GTA TTA AAG GCA ACC AGC GTA GAG GCG AGA GAA AAA ATA GIC TTT CAG GGT GAG CAA GAG AAA CCA AAA GAA GGA ACA AAA CIG ATT GIT GAG GAA ATG GAA TTT GAG ACA GAC ACA GCC GCC GGA GAG ATT GTC GAA CAA AAG GAA AAG ATA GCC GTT GGA GAC ATT CTA CAA CTT ACA GAT GGG AAG AAG AGG ACC GGA AAT CTC GAC ACT ATA GCA GTT CTA GAA AAT GAT AGC CIC TTA ATA AAC ATA GAC ACP ACT CCG GCT CCA 432 540 486 378 324 270 216 162 108

KOD DNA POLYMERASE - (PCNA) fusion protein (Fig. 17-EE)

CGAGAGCATC CGGCAGAGCT ATGAAGGAGG CTCTGGGACG TCTCCCGCTC ACCGGCGAGA GCCGTCTTCG GTCAGCCGAA ATTCAGAGGA TGGGCGACAG AAAGACCCGG ACGTTCTCAT AACCTACAAC GTTGACGTCG AGCTACGCCG ACGAGGAAGG GGCCAGGGTG ATAACTTGGA GACATTGAAA CTCATAGACA AGGGATTAGT GCCAATGGAA GAGGTCTGGA AACTCTACTT TACTCATCCG ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE ATAACGATGA AGGGCGCGCT TACAGGCAGA CAGAAGATAA GATACGCTCA GCCTATGAGA GAGCTTGGGA TATCCTGTGA AAGCGCTGTG ACCGACGGAT TTCTGCAAGG GTGTACCTAG AGAAGAAGAT AGGAGTTCCT TCCGATGGAG ACCTTGAGAG AGTCGCCCGC TAAGACGGAC AAAAGCTCGG AATAAACTTC TCTCGACGGA GAGGGAGATG CTCTCTACCA CAGCAGTTAT TGACATCTAC GGAATGAGCT TTTTTGCCAC CCATCAAGGA GGTACTGCAA GGGCCATCAA ACTTCCCAGG ACAGAGAAGG ATTTTAGATC GATAGAGGAA GGAGTGTGCA GATCCTGGCA AACAGCTACT ACGGTTACTA GAAGGCCACG ATTTATCCCG ATGCAAGGAA CTATGTAAAA GATAAACCTG GTTTGCCGTC TGAGGGCGAG AATACCTGGA CCTGTACCCC GGCCCCGAAC CAGCACTGGC GGAGAAGGTT GGCGACGAGG GAGTACGACA CCCACATACA ATAAAGCGCT GAGTTCGCCG CAGGACXXXC GCCGATGCTG AAGTACGGCT GAGAGCGTAA ATTGACCCGA AGCCTGCTTG GAGACCTCCT TATGACGTTG GAGCCCGAGA AAGCCCGATG AACCTCGTTG GCCCAGCTTT TACTCGATGG TACGCTGAGG GAAGTGAAGG GCCCTCGGAA GGCGACAACT TCAATCATCA CCCCACAGGT GAGGGTTGTG AAAAGGAGCT AGTGGTTCCT AAGATGCGAA GGTCACATAC CGCTTGAGGC GACGGATACA CTTCGATCTC GGGATGGAAG TCGACTTCGC TCCTCCGTGT AGAACGTGGA TCTCCCCTAC AGGGGCCAAT CCTTATGATA AGCTGAAAAT GCTCGCCTTC TACCCTTCGC CAGCGATAAG GGACAAGATA AGTTCCTCGG GAGACCAGTT TAACCGCCGA GAGGCACGGG TTGAACCCTA CTTCTACGCC CTGTCATAAG AATTTTCAAG TCGAGAGGAA TCACCCACAA CTCGCTTAAT AAATAACCAC CGGCCTGGGG AAACCGTCAA TTAAGGTAAT AGCCTGGGAA CGGCCAGTCC CGAGCCGAAG CAAGCGCTAC AAAGAAGGCT CTACAGCGAC CGGCTATGCA GCTCCTCGAT AGAGGAGAGG CGGCCACCGC CGTCTCGCCG GGAGAACATA GGCCAGAAGA CCTCAGGAAG CGTTTATGAA CTATCTGAAA TGTGAAGGAG AAGGGAGTAC 660 900 840 780 720 600 540 480 420 360 300 1020 960 240 1440 1260 1200 1140 1080 1680 1620 1500 1380 1320

ATGGAGTTCC

TCAAGTATAT CAACGCCAAA CTTCCGGGCG CGCTTGAGCT

CGAGTACGAG

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GGCAAGATAA
                                                                                                                                 GACCCGACGA
                                                                                                                                       TACATCGTGC
                                                                                                                                                GCCGTTGCCA
                                                                                                                                                        GTGATCCACG
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                                                                                                                                                                        GAGACGCAGG
                                                                                                                                                                                         GGCTTCTACA AACGCGGCTT
                                                                                                                 AGACAGGTTG
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                 TAT
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                                                                                                         TTT GAA
                         GAG
 AGA
        AAG
                 AGC
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                                                                                                                               CGACGCCGAG TACTACATTG AGAACCAGGT
                                                                                                                                                CGCGAGAGGA
                                                                                                                                                                                        CTTCGTCACG
                                                                                                                        CTTCGGTTAC
                                                                                                                                        TGGGAGGATA
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                                                                                                                                                                CGAAAAGCTG
                                                                                                                                                                        TGAAGCTTTG
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                                                                                        CCA
                                                                                                        TTT GAA GGT GCA AAA
                                                                         ATC
 TTC
        AAT
                                                                 AAC
                                                                                 TAT
                                                                                               GAT GAG GCC
                TCC
                         TTG
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CTA
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                GAC
                         GAC
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                                                                        CTA
                                         AGT
                                                                 TTC
                                                                                                                       CGCAAGGAAG ACCTGCGCTA
                                                                                                                                       GGCGACAGGG
                                                                                                                                                GTCAAAATAC
                                                                                                                                                        AAGGACTACA
                                                                                                                                                                AGCAAGTACG
                                                                                                                                                                        CTAAAGGACG
                                                                                                                                                                                GTGAGGCGTG
                                                                                                                                                                                         AAGAAGAAGT
                                                                                GIT
        ATG
 CIG
                ATG
                                         GAC
                                                 GTA
                                                        GAT
                                                                 TTA
                                                                        AAG
                                                                                         AGA
                                                                                               GCG
                                                        GTA
        CCC
                                                                 GAG
                                                                        AGA
                                                                                GTT
                                                                                        GTT
                                         AGC
                                                        GAA
                                                                                GAA
                                CAG
                                                                ATA
                                                                        GGT
                                                                                        GTC
                                                                                               TTT
                                                                                                                                       CGATACCGTT
                                                                                                                                               GCCCTGGAAC
                                                                                                                                                       AGGCAACCGG
                                                                                                                                                                AGGTTCCGCC
                                                                                                                                                                        GTGACGTCGA
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                                                                                                                                                                                         ATGCGGTGAT
AGA
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                                                                                CCA
                                                        GAG
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                                                                                        CTG
                                                                                                AAA
                                                                                                        GAG TTT
                                                                                                               2325
        ATG
                         GAG
                                                                        GCA
                                                                                GAA
                                                                                                GTT
                                                GAA
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        GAG
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                                                                CAA
                                                                                ACA
                                                                                        GAC
                                                                                                ACA
                                                                                                        GCC
                                                                                                                        CCAGAAGACG
                                                                                                                               TCTCCCAGCC
                                                                                                                                                                                GATAGCGAAA
                                                                                                                                                                                        AGACGAGGAA
                GGA
                                GAG
                                                                        AAG
                                                                                                                                       CGACGAGTTC
                                                                                                                                                       TCCCCACGTT
                                                                                                                                                                GGAGAAGCTG
                                                                                                                                                                        GAAGGCCGTG
                                                                                                                                               GGTGATAAGC
GAG
        TAT
                                                                GGA
                                        GCC
                                                        GIT
                                                                        GAC
                                                                                                        CAA
                AAG
                        ACA
                                                                                        CTA
                                                                                                GAA
       TAC
               CCC
                        AAG
                                                AAA
                                                                ACT
                                                                                GGA
                                        AGG
                                                        GAC
                                                                        ACC
                                                                                        AAT
                                                                                                GAT
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                                                                                                                                2220
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                                                                                                                                               2100
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                                                                                                                                                               1980
                                                                                                                                                                        1920
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                                                                GCA
                GAT
                        AGC
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                        GCA
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                                                GCI
                                                        CCA
                                                                        ATA
                                                                               AAC
                                                                                       CCG
                                                                                               ATA
               648
                        594
                               540
                                       486
                                               432
                                                       378
                                                               324
                                                                       270
                                                                               216
                                                                                       162
                                                                                               108
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(PCNA) - KOD DNA POLYMERASE fusion protein (Fig. 17-FF)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

		TCA A			ATG CCA
AG AAA	_	AGC ATA			TTT GAA
GGA	CTA	TTT	GCC		ATC
GAG			ATG		GTA
		AAA '			TTT
		TAT (_	GAA
	CTA I			_	GGT
		GTT 0		9 229	GCA
GAG A		GTT G	GTT G		AAA
ATA A		GAA C			GAG
ACA A		CCA G			TTT
		GAA A			GCC
CAA G	_	ACA A	GAC C		CAA
3GA A		ATT G		_	CTT
ACT G	ACC T	GGA G	AAT C	_	ATA
GCA A		GTT A	CIC C		GAC
ACA	ATA	AAC	CĊG	TA	
324	270	216	162	108	54

Fig.17 FF (cont)

432 486 540 594 648

ATAACGATGA GATACGCTCA GTGTACCTAG CGGCAGAGCT ATGAAGGAGG GCCTATGAGA CTCTGGGACG TCTCCCGCTC GAGCTTGGGA AGGAGTTCCT TCCGATGGAG GCCCAGCTTT ACCGGCGAGA ACCTTGAGAG AGTCGCCCGC GCCGTCTTCG GTCAGCCGAA ATTCAGAGGA TGGGCGACAG AAAGACCCGG ACGTTCTCAT AACCTACAAC GGCGACAACT TCGACTTCGC GTTGACGTCG TCTCGACGGA GAGGGGAGATG ATAAAGCGCT TCCTCCGTGT AGCTACGCCG ACGAGGAAGG GGCCAGGGTG ATAACTTGGA AGAACGTGGA GACATTGAAA CTCTCTACCA TGAGGGCGAG GAGTTCGCCG AGGGGCCAAT CGAGAGCATC CAGCAGTTAT TGACATCTAC GAGTACGACA TACCCTTCGC GAGGTCTGGA AACTCTACTT TACTCATCCG CAGGACXXXC CAGCGATAAG AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT TTGAACCCTA CTTCTACGCC 120 ATGGAGTTCC TCAAGTATAT CAACGCCAAA CTTCCGGGCG CGCTTGAGCT CGAGTACGAG ACCGACGGAT TTTTTGCCAC AGGCCCCCT TACAGGCAGA TTCTGCAAGG TATCCTGTGA TAAGACGGAC AAGCGCTGTG AAAAGCTCGG AATAAACTTC CTCATAGACA AGGGATTAGT GCCAATGGAA GGCGACGAGG AGCTGAAAAT ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA AGTTCCTCGG GAGACCAGTT 240 CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA TAACCGCCGA GAGGCACGGG //ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC CTGTCATAAG AATTTTCAAG 60 AGA GAA GAT AAA GAT GCC CCATCAAGGA GATAGAGGAA AAGTACGGCT TTAAGGTAAT CTACAGCGAC GGTACTGCAA GGAGTGTGCA GAGAGCGTAA CGGCCTGGGG GGGCCATCAA GATCCTGGCA AGAAGAAGAT GAAGGCCACG ATTGACCCGA TCGAGAGGAA GCTCCTCGAT ACTTCCCAGG ACAGAGAAGG ATTTTAGATC GGAATGAGCT ATA ATG ATA AAG GTC AGC GAG TAT GGA AAG ATGCAAGGAA TATGACGTTG CCCCACAGGT GGCCCCGAAC AAGCCCGATG AAAAGGAGCT GATAAACCTG AATACCTGGA GCCGATGCTG AAACCGTCAA AAAGAAGGCT ATTTATCCCG CCTGTACCCC CTATGTAAAA GAGCCCGAGA GAGGGTTGTG CAGCACTGGC GGAGAAGGTT GTTTGCCGTC GAAGTGAAGG GACGGATACA GCA TTA TCC TTG GAG GTG AAG GAA GAC GAC GGA GIT AGT GAC AGC ATA AACAGCTACT TCAATCATCA TCACCCACAA AACCTCGTTG AGTGGTTCCT TACTCGATGG AAGATGCGAA GCCCTCGGAA GGGATGGAAG AGCCTGCTTG TACGCTGAGG AAATAACCAC CCCACATACA ATC GAA ATG CCC ATG ATG GTT AAA GTA ACC GAG GTT GIT CIT CAG GAGACCTCCT ACGGTTACTA CTCGCTTAAT CGCTTGAGGC GGA CAA GAA GGA AAA GIT GAG ATG TTT GAA AGAGGAGAGG GGAGAACATA CTTCGATCTC AAGGGAGTAC CGGCTATGCA CGGCCACCGC CGTCTCGCCG GGCCAGAAGA CCTCAGGAAG CGGCCAGTCC GGTCACATAC AGCCTGGGAA CGTTTATGAA 840 CGAGCCGAAG CTATCTGAAA 660 TGTGAAGGAG 600 TCTCCCCTAC CCTTATGATA 480 GCTCGCCTTC 420 CAAGCGCTAC 360 GGACAAGATA GAA GAG GGA GAG GAG GTC ATT GCC AGG . GAG ACA AAG GCC ATA AAG TAC AAG AGC AAA 1080 1440 . 900 780 720 540 300 1500 1380 1320 1260 1200 1020 960 1620 1140 GAT CIC ATT CTA ACT GAA AAT GAT GCT GCA AGA

GACCCGACGA AGCACAAGTA GTGATCCACG AGGATAGTCA AAGAAGTTAC CGAAAAAGCTG AGCAAGTACG AGGTTCCGCC GGAGAAGCTG GAGACGCAGG GGCAAGATAA CAACGCGCGG ACTTGAGATT GTGAGGCGTG ACTGGAGCGA GATAGCGAAA AGACAGGTTG GTTTGAGTGC GTTGAGAGAA TTCTGAGAGC TACATCGTGC TCAAGGGCTC TGGGAGGATA GGCGACAGGG CGATACCGTT CGACGAGTTC GCCGTTGCCA GGCTTCTACA AACGCGGCTT CTTCGTCACG AAGAAGAAGT ATGCGGTGAT AGACGAGGAA 1800 CGAGGGTTCT TGAAGCTTTG AGAGGTTGGC AGCAGATAAC CGACGCCGAG TACTACATTG AGAACCAGGT TCTCCCAGCC TTGGCTGAAG CCGAAGGGAA CT //TAG 2325 GAGGGATTTA AAGGACTACA CTTCGGTTAC CGCAAGGAAG ACCTGCGCTA CCAGAAGACG 2280 CGCGAGAGGA GTCAAAATAC CTAAAGGACG AGGCAACCGG GCCCTGGAAC GTGACGTCGA GAAGGCCGTG GGTGATAAGC TCCCCACGTT 2220 1980 2160 2040 1920 2100 1860

(PCNA) - Vent DNA POLYMERASE FUSION PROTEIN (Fig. 17-GG)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC TAT GAA GIT GAA AGA ATG TCA AGC ATA TTT AGC ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA AGC ATG AGG GCC ATG GAT CCA AGT AGA GTT GTC CTG ATT GAC CTA AAT CTC CCG AAA GAT GCC TCT ACA TTT ATA ATG AAG GAC CAC CTA AAG GAA GGA AGA CTT ACA TTC CTA CTG GCT CCA AGA GTT GAA GAG ACA ATA AAG TTT GGA GTC AGC TAT GAA GAT GAG GGA CTT CCA AAG AAA GGA GAG TTT AGA GTT TTC ACT CTC TTA GCA CTA GCA AAG CCC GAA AAA ATC GTG TTG GAG TAT GAA GTT GTT GAA CCA GAA ACA ATT GGA GTT AAC AAT GAA ATG CCC ATG CAA ATG TCC GAC ATG GTT AAA GGA CTT AAG AAC CTA ATA GAC GGA GTT TTC AGT GAC AGC ATA AAA CTA AAG AGA GGT AAA GCA ATC GAG GTT CAA GAG TTA GAG ATA ACA GAA ACC CAG GAA GTA GTT CTT GGA GAT GTA GAA GAG GTT ATG ATT GAA GGA GAG GAG GTC AAG GAC ACC TTA ATA GAG ATT GCC AGG GAA AAT GAA GTT CAA GGA ACT GCA ACA TAT ACA ATA AAG GCC CTA AAG AGC GCA AAG TAC AAA GAT GCT GAC CTC CCA ATT AGA GAT GAA CTA ACT 54 594 540 486 432 216 162 108 378 324

ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CTATAATCCG AATTTTTAAG 60 AAAGAGAACG GGGAGTTTAA AATAGAACTT GACCCTCATT TTCAGCCCTA TATATATGCT 120 CTTCTCAAAAG ATGACTCCGC TATTGAGGAAG ATAAAAGGCAA TAAAGGGCGA GAGACATGGA 180 AAAACTGTGA GAGTGCTCGA TGCAGTGAAA GTCAGGAAAA AATTTTTTGGG AAGGGAAAGTT 240

ACAGAATACG GAAAAGCTTG ATAGCTAAGG AGACACTACA GGGTATCCTA GCAATGAGGC GGATATAGGT GTATCCCCAG AGGGTGGCAT ATAATAAGCT CCTCATGTCG GATGAAGAGG GAGTATGAGG AAGAAAGCCA TATGCGGACA ATGCTCGATT GAAAATATCA CGGCGCTTAA TTGCCGGCAG AAAGCTGTAG CAAAGTGTAT ACGTATGAGC TGGGAAACAG TATGAAGCAG GATCTTTTCC CCCAAGATTC AAACGGGCAG AAAGACCCCG GTCGATGTTG AGTTATGCCG GATATTGAAA CTCATAGACA GAAGTCTGGA TACTTAGGAT ATCCTAGAAA ATATCGTTCT AGGAATTCCT ATAGGCAAAG GAACAACTTA GGGACGTCTC AGCTCATTTT GCAGGATAAC GCTTTTACTT CTGACGGCTT TAGAGATGAC AGGCAAGATG ATACCCTTGA ACGCGAGGAA AAGAAAGCAT AGAGGATGGG AAAAGCTGGG AGGGCTTGAT CAGCIGIGGI CGATAGCAAA AAGTTGTTAG AGACTCAGGC AAGATATAAA TCTGCAAGGA CAGTTGTGCG ATGTGATAAT TGTCCAATGA ATGAAGAAGA CGTTTTATCA TTTATTTGGA TCGGGAAGGA TTTTAGGAAA TTATCCATGA ACTCGAAGCG ACACAAGTAC CAAAGGGAGC AAGACTTGCC GCAGATTACC AGATGTTGTA AAAGGTTTTA AACAAGGGGC GAGAGGATTC AAACTACATA TTATGCCACA GATAAGAGAA GTACTCGAAG GGCTATTAAA GAAGAAAATG CTTTCCGGGC AAAAGAGGGC TTTCCGCAGT CCTGGGAGGA TGAACTTGCA GAGATCAAGC ATTCTTCCCC GAAAAAACTA AACCAAAAGC AAGGACGATA AGTTCGGCTT AACTTACAAT AAGAGAAATG GGCCAGAGTA TCCCATGGAG TGACATTTAC CGAGCATCCC TGATAGTTTT TGAGGGAGAT ATAGAGGAAA GATCCGGACT AACTCCAAAC ATACCCGGGG GAGAAAATAG GAGGCTATAC GAATGTGCTG AAATCCACAA CTGTACCCTT TATGTAAAAG CCGAACAAAC ACCGGCAACC GCCCAGTACT AACCTCCCAA GCTGTGGAAA GGGGACAATT GAATATGACA CAAGACXXXC TTTGGATACA GGAAAGATAA GCAAGAGGGA AGGGATTTAA TTGGAAGTAG TTTGTTACAA TTGCTTGCAA TTTATTCCCT TGTAAGAATT ATGGAAGCTG AAATTAGGAG GTCTTAGGAA ATAAAGCGTT ATCACATGGA GAATTTGGAA GGAGACGAGG GAAAGGAGGA ACTACATAGA GCGATAGGGT AGGACTACAA AAAAGCGCTA AGTTCGGCTT AAAGCGTTAC CAATAATAGT CGTATACGCT TAAAAGTGAA CAAAATACAG TTAAAGAGGG TTCCAGGTCT AAAAGCCTGA ACAGCTATTA CCATACTCGG ACGATGTTGC CAATGGAAGA CAGAGGAAAT TCAAGGGTAG GGGACAAAGA AGCTTAAGCT CAGCTATGCG TAAGGAGAGA TTGACCCGAT AGCCAGAAAA CTGATGAGGA AGCTGGCAAA TTGATTTGCC AAAATATCGA AGGGCGAGAT TACCCTTTGC TCGTGGAGTG TTGTTCAAGT GCTTGAGCTT AAACCAAGTT AATTTTACTT ACCGGGCACA AGCCATTGGC GGTTCCACTT AAGTGTTGAA TTGGAGTGAG TGCAGTCATA ACTCATTAAA TAAGGTTCTT CGCATGGGGG CGGCTATATG CGAAAAGAAA GGACTTAATT TCCGATAGTA TACTCACAAC AGGTTTGTGG AGAGTATAAA GTATCTTTTA GCTGATAGGT TGCTAGGGCA TGCCGCTATA TGAGGCAGTT AATCCACTTT ACATCCCGAA GTATCTCATA AATAATGATT CCTTGCCTTT CAAGCGTTAT GGGCAAAATA TGTTAAAGAA TTTGCCGTAT 1980 1920 1740 1620 1560 1320 1260 540 48C 2040 1860 1800 1680 1440 1380 1200 1140 1080 1020 960 900 840 780 720 660 600 2100 1500

Vent DNA POLYMERASE - (PCNA) FUSION PROTEIN (Fig. 17-HH)

CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GGTAG 2325

Fg. 1) HH (CONT)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

GAGTATGAGG AAGAAAGCCA GTATCCCCAG GAAAATATCA AGGGTGGCAT ACGTATGAGC TATGAAGCAG CCCAAGATTC AAACGGGCAG AAAGACCCCG GTCGATGTTG AGTTATGCCG AAAGAGAACG AGACACTACA GGGTATCCTA ATGCTCGATT GCAATGAGGC GGATATAGGT CGGCGCTTAA CAAAGTGTAT TGGGAAACAG GATCTTTTCC GATATTGAAA CTCATAGACA AGGGAACATC GAAGTCTGGA AAAACTGTGA TATGCGGACA CTTCTCAAAG ATGATACTGG ACACTGATTA CATAACAAAA GCAGGATAAC GCTTTTACTT CTGACGGCTT AGGCAAGATG ATAGGCAAAG GAACAACTTA GGGACGTCTC AAGAAAGCAT ATGTGATAAT ATGAĂGAAGA CGTTTTATCA CAGCTGTGGT GAGTGCTCGA GGGAGTTTAA AATAGAACTT AGGAATTCCT TAGAGATGAC ATACCCTTGA ACGCGAGGAA TCGGGAAGGA TTTTAGGAAA CAGTTGTGCG AGAGGATGGG AAAAGCTGGG TGTCCAATGA ATGACTCCGC AAGATATAAA TCTGCAAGGA TTTATTTGGA AGGGCTTGAT AGCTCATTTT GAAAAAACTA GAAGAAAATG GAGATCAAGC GGCCAGAGTA AAACTACATA AACCAAAAGC AACTTACAAT AAGAGAAATG AACAAGGGGC GAGAGGATTC TTATGCCACA GGCTATTAAA TGAACTTGCA ATTCTTCCCC TGAGGGAGAT CGAGCATCCC TATTGAGGAG GATAAGAGAA GTACTCGAAG CTTTCCGGGC AAAAGAGGGC TTTCCGCAGT CCTGGGAGGA AAGGACGATA TGATAGTTTT AGTICGGCTT TCCCATGGAG TGACATTTAC TGCAGTGAAA ATACCCGGGG ATAGAGGAAA CCGAACAAAC ACCGGCAACC GCCCAGTACT GCTGTGGAAA ATAAAGCGTT ATCACATGGA GAATTTGGAA GACCCTCATT GATGGCAAGC AACTCCAAAC GAATGTGCTG TTGCTTGCAA AAATCCACAA CTGTACCCTT TATGTAAAAG ATGGAAGCTG AAATTAGGAG GTCTTAGGAA GTCAGGAAAA ATAAAGGCAA GGGGACAATT GGAGACGAGG GAATATGACA TTTGTTACAA TGTAAGAATT AACCTCCCAA CAAGACXXXC TTGGAAGTAG TTTATTCCCT TAAGGAGAGA AAAAGCGCTA TTCCAGGTCT AGTTCGGCTT ACAGCTATTA TTGACCCGAT CCATACTCGG ACGATGTTGC CAATAATAGT AGCCAGAAAA CTGATGAGGA TCGTGGAGTG AGCTGGCAAA CAATGGAAGA CAGAGGAAAT CGTATACGCT TCAAGGGTAG GGGACAAAGA TTGATTTGCC TTGTTCAAGT AAAATATCGA AGGGCGAGAT AGCTTAAGCT TACCCTTTGC AATTTTTGGG TAAAGGGCGA TTCAGCCCTA AAAAGCCTGA AAAGCGTTAC CAGCTATGCG CTATAATCCG CGAAAAGAAA GTATCTTTA TGCCGCTATA GTATCTCATA AATTTTTAAG GCTTGAGCTT ACTCATTAAA CGGCTATATG GCTGATAGGT TGCTAGGGCA TGCAGTCATA TAAGGTTCTT CGCATGGGGG TACTCACAAC AGGTTTGTGG AGAGTATAAA TGAGGCAGTT AATCCACTTT ACATCCCGAA AATAATGATT CCTTGCCTTT TATATATGCT GGACTTAATT TCCGATAGTA TGTTAAAGAA TTTGCCGTAT CAAGCGTTAT GGGCAAAATA AAGGGAAGTT GAGACATGGA 600 1440 ·1140 1020 960 900 840 660 540 480 360 1500 780 420 1680 1620 1560 1380 1320 1260 1200 1080 720 240

ATAATAAGCT ATATCGTTCT CAAAGGGAGC GGAAAGATAA GCGATAGGGT AATTTTACTT 2160 AAAGCTGTAG CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GG 2325 // ACAGAATACG ATCCTAGAAA ACACAAGTAC GATCCGGACT ACTACATAGA AAACCAAGTT 2220 CCTCATGTCG CGATAGCAAA AAGACTTGCC GCAAGAGGGA TAAAAGTGAA ACCGGGCACA GAAAAGCTTG TTATCCATGA GCAGATTACC AGGGATTTAA AGGACTACAA AGCCATTGGC ATAGCTAAGG AGACTCAGGC AAAGGTTTTA GAGGCTATAC TTAAAGAGGG AAGTGTTGAA TTGCCGGCAG AAGTTGTTAG AGATGTTGTA GAGAAAATAG CAAAATACAG GGTTCCACTT TACTTAGGAT ACTCGAAGCG TTTGGATACA GAAAGGAGGA TTTAAGGTAT 2280 2100 2040 1980

ATG CCA TTT GAA ATC GTA TTT GAA GGT GCA AAA GAG TTT GCC CAA CTT ATA GAC GAT GAA GGA AGA CTT ACA GAA GAA ACC GCA AGT AAG TTA ATA GAT GAG GCC GCG TTT AAA GTT ACA GAA GAT GGG ATA AGA CIC ATG TCA AGC ATG AGG GCC ATG ACA ATA AAG TTT GGA GTC AGC TAT ACA GAA TTT ATA ATG AAG AAA AGC ATA TTT AGC CTT CCA TTC ACT AAG AAA SGA GAG GAC CAC CTA AAG GAT GAG GGA GAT GCC TCT TTT AGA GTT GCA GCA CCC GAA AAG AAA GAT CCA AGT AGA GTT GTC GAG ATC TAT GAA GTT GTT GAA AAT GIG AAG AAC GAA GAC GGA GTT GAC AGT GAC AGC ATA ATA TTC CTA AAG AGA GGT GTA ATG GTT AAA GGA TTA GAG ATA ACA ATC GAG GTT CAA GAA ACC CAG GAA GAT GTA GTT CTT GAA GAG CTG AAA CCA AAA GCA GAA GAA ATT GGA GAG GTC GAA GAG GAG TAT GAG ATT GAA CAA GGA ACT GCA ACA AAG GAC ACC TTA ATA ACA ATT GGA GTT AAC GAC CTA AAT CTC CCG GCC GTT AAG GCC ACA ATA GAC AAG TAC AGG AAG AAA GAT GCT GAT GAP CIC AGC GCA CTA ACT GAA AAT d C C 5 4 594 648 540 432 486 378 324 270 216 162 108

eep Vent- (PCNA) DNA polymerase fusion protein (Fig. 17-II)

GAGGTATGGA GGCTGTACTT TGAACACCCT CAGGACXXXC CCGCAATAAG GGATAAGATA V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGTACGACA TTCCGTTCGC GAAGAGGTAC AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA AAAGAAAACG GCGAGTTTAA GGTTGAGTAC GACAGAAACT ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) AGTTCCTGGG GAGGCCGATT TAACCGCCGA GAGGCATGGG TTAGACCTTA CATTTACGCT CGATTATAAG GATTTTCAAG 360 300 240 180 120 60

(cont)

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GTAAAGATAG
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// ATG

CCA

TTT

GAA AAG TTA ATG

ATC

AGT AGG

AGC ACC

ATG GCA

GCC

AAA GAT ATA GTA

TAT CCA GAT TTT

GAA GTT AGT AGA GAG GCC GAA GGT GCA

GAA GIC CIG

CCA

GAA

ACA

ATT CTA

GGA

ATT

GAC ACA

AAT CTC CCC

162 108

GTT

GAA GAT GGG ATA

ATA GAC

GCG GIT

AAA GAG TTT AAA

TTT

GCC

GIT GIT TAT CTTGAA GAA AGA ATG AAG GAC ACA ATA AAG GGA GTC AGC GAA GAT GAG TTT AAA GAT GCC TCT CTT ACA CCA AAA CAC ATA ATG TTT AGA TTC GGA TAT GGA AAG GAG AAG GCA GGA CIC GCA CCC TTA CTA GAA AAG AAT TTG GAG TTC TCC GIG AAG CTA AAC ATC GAC GAC GGA ATA GAA ATG CCC ATG GTT TTC AGT GAC AGC GAA ACC CAG GTA ATC GAT GTA GAA ATG GTT AAA AAG AGA TTA GAG GAG GTT GTT ATA CTT ATA GGT CAA GGA CAA GAA AAA GAG GGA ACA AAA ATG GIT CTT GAG TTT GAA ATG GCA GTT GAA GAG ATT GAG GGA GAG GAG ATT GAA GTC AAG ACA AAG AGC GCA GCC AGG GAA TAT TAC ATT AGA AAG GCC GAT GAA ATA AAG CTA ACT GIT GAC CTC GGA ACT GCA ACA GAC ACC TTA ATA CTA AAA GAT GCT AAT CCA 648 702 594 540 486 432 378 324

(PCNA) - Deep Vent DNA polymerase fusion protein (Fig. 17-JJ)

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V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

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			• •	-			-	• •	4-	-	• -	`-	CCA
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GGA	ATA	GTC	GAT	ATA	GAT	CCA	TTT	AAA	CAC	ATA	AGG	AGT	T GAA
AGA	AAG	AGC	GAG	ATG	GCC	TTC	AGA	GGA	CTA	TTT	GCC	AAG	AA ATC
CTT	TTT	TAT	GGA	AAG	TCT	ACT	GTT	GAG	AAG	AGC	ATG	TTA	IC GTA
ACA	GGA	CIC	TTA	GCA	CTA	GCA	CCC	GAA	AAG	ĀĀĀ	GAT	ATA	-
TTC	AAT	TCC	TIG	GAG	GIG	AAG	CTA	AAC	ATC	TAT	CCA	GAT	TTT GAA
CTA	GAA	GAC	GAC	GGA	AGT	GTT	ATA	TTC	CTA	GAA	AGT	GAG	AA GGT
CIG	ATG	ATG	ATC	GAA	GAC	GTA	GAT	TTA	AAG	GTT	AGA	GCC	3T GCA
GCT	CCC	GIT	GAG	ACC	AGC	GTT	GTA	GAG	AGA	GTT	GTT	GCG	CA AAA
CCA	ATG	AAA	GIT	CAG	ATA	CTT	GAA	ATA	GGT	GAA	GIC	TTT	AA GAG
AGA	CAA	GGA	CAA	GAA	AAA	GGA	GAG	ACA	AAA	CCA	CIG	AAA	AG TTT
GTT	ATG	CIT	GAG	GIT	TTT	GAA	ATG	ATT	GCA	GAA	ATT	GTT	TT GCC
GAA	GAG	GGA	GAG	GAG	ATT	GTC	GAA	CAA	AAG	ACA	GAC	ACA	C CAA
GAG	TAT	AAG	ACA	ATA	GCC	CTA	GIT	GGA	GAC	ATT	CTA	GAA	AA CTT
	TAC	GCC	AAG	AAG	AGG	AAA	GAC	ACT	ACC	GGA	AAT	GAT	CT ATA
	ATT	GAT	AGC	CTA	GAA	GAT	CIC	GCA	TTA	GTT	CIC	GGG	'A GAC
	AGA	GAA	GCA	ACT	AAT	GCT	CCA	ACA	ATA	AAC	CCG	ATA	ń
	702	648	594	540	486	432	378	324	270	216	162	108	54

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AAAGAAAACG GCGAGTTTAA GGTTGAGTAC GACAGAAACT TTAGACCTTA CATTTACGCT //ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG 60

10.4/1860

Fig 17JJ (cont)

GCCCTAGAGT GACACAGATG GCAAAAGCCC GATTACAGGC AGGCAAGAAA AAGTTCTGCA CCGGATACGC TTAGTTTCCC CTAAGGGAGA GCCTACGAGA CTGTGGGATG GAGCTCGGTA ACTGGAAAGG GCAATCTTCG TACCACGTGA ATGCAGAGGC AAAGATCCCG GTCGAGGTAG AGCTATGCTG GACATAGAAA AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGTACGACA TTCCGTTCGC GAAGAGGTAC GCCGTTCTTA TTCGATCTCA GGGTACATAG GITGCCGTGG CTAGTTATTT GTAAAGATAG AAAGAAACCC GAAGGGAAGA GAGGGCTTCT TATATAGAGT AAGAGGGCCG CTAATAGACA GAGGTATGGA AAGATAGTGA CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGG ACTAAACAGA GAATATTAGA TCGTAGATTA TCGTAAGGAA GGAATGAATT GAATTATAGA CAGGTCTTAC GGAAGCATAA TGCTGAGGGG CAAAAAGGTT ACGAGCAGAT TAATCACTAG ACGTGAGAGG GACTCTACGC GTTGGTACTG AACGGGCAAT TAAAAAGGAA AGGACTTCCC TGAACAGGGA TAGATTTCAG GCTACGCTGG GACTGGAGAG GAAAGCCAAA TTGGGGATAT AAAAGCTCGG ATGTTATAAT TTTCCAGCGA CCCTCTATCA AAGGCCTAAT GGCTGTACTT TTAAGGAGGT AAGCAAAAGT TTTCTAGGTC GGGAGTTCTT TTAGGAGAAC ATGAGGAAGA CAAAATCCTG GCAAACAGCT GAGCCTGTAC GGAGAAAGTT GACAGCGGTG GAGATAAAGG GAAGGATACA TACCTACAAC GGCGATTCTT TCGACCTTCC GTATGACGCT AGACGGGCCA AGCCGCTAGA CACGAGGCCC AACTGAAAAG GGGGCTTGAA GTTCTTCGTG TATAAACGCC CACAATTCCT GGAACTGGAG GAAAAGTTCG TAAGGAGTGC GATGAAAGCT GGGGTTTATC AGGGTGTAGG GGGATACGTT GGCTCCAAAC CCCAATGGAG AGTTGCAAAG GATAAACCTC GATAAAGCTA CCCCTGGGAA GGGACGGTAG GAGGGAGATG AGCCAAAGTC CGAAGGGGAG TCCAATGGAA GGCGATGAAG TGAACACCCT TGCCGAAAAG GTAAGGAAGA AGTTCCTGGG GAGGCCGATT GGCATGGCTT GGCCTTTGGG CCTAGAGGCT TTCAACTGGC CAGGACXXXC CCGCAATAAG AAGGAGCCGG GAGTATTACA ATAAGCAAGA GGAGTAAAGG ATAGTCAGGA GGGGCAAAAC GCAGAGAGCG GAATACGATG CCCTCGATAA GCCCAGCTTT TACGCTCACG GAGTTCGCGA TACAGGAAAG CTTCACGAGT ACAAGGCTAT CTGAGCAAGT ATCCTAAAGC ACGAAGAAGA AAGCTCCCAG TCTAAAGACC CCCAGCCTGC AAGCCGGATG AACTTGGTGG TATTCAATGG CCAACATACA ATAAAGCGGT TCCTCAAGGT ATAACGTGGA AAAAGATCGA TCTCCCGTAC AACATCAAGA CAAGGTTAGT AAGACCTCAG GGGCTATCCT GGGACTGGAG AGTATGCGTT GGCTGTTGGA GGTTCAAAGT TTACGGCCTG ATTATGGGTA CAATCGAGAA GAAGATGCTT TCAAGAGGTT TCGCCCCAGA TAATCACCCA AGAAAGGGCT AGAGGGAGTA AGTGGTACCT CCTCAGGAAG AGGATGCAAA AGATAGCTGA CCCTCGAGGC AGGGGCCCAT AGCTCAAGTT AGAAG TAA TAGAAAATCA TGAGGCCTGG ACGAAATACC ATGGCAACGT CCGAGGAGAT TATAATGATA GGATAAGATA TTATGGGTAC GGTTGGGCAC CTGGGAGGG CGAGAGAAGG CGGCCAGCCC GGTAACGTAC GGCCTGGGAG AGTTTATGAG CTTTGACCTC TGAGCCAAAG CTATCTAGTT GATAAGGGAG GCTCGCATT GTGGCAGAAG TGCAGAGGAG AGGTCCGCAC TGAGGAGGCA CGAAATAGCC GATAGATGAG GCTTGAGTAC AAAGAAGAAA CTTATACATA GGGGAGGGAA ATTGGATGAA TAACGTCTCA GGTTTTACCI CATGGTGATA TCCAGAAAAG 1140 1080 2220 1980 1920 1860 1800 1680 1620 1560 1500 1380 1320 1260 1200 1020 2160 2100 2040 1740 1440 960 900 840 780 660 600 480 720 540 420 360

105/186

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

 $\tt GTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCACGAAGCACAAGTACGATG$ $\tt GTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAgcGTTTGGCCGCCAGAGGT$ ${\tt CGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGCGC$ $\mathtt{ACTGGAGCGAGATAGCGAAGGAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAA$ ${ t GAACTCGAATACGAGGGCTTCTACGTCAGGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCCAAGATAACCACGCGCGGGCTTGAGATAGTCAGGCGCG$ $\tt CTATGCAGACAGACGGTCTCCATGCCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTC$ A TGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAAAGTCCT ${ t CAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCGCCATCAAGATTCTCGCCAACAGCTACTACGGCTACTACGGCT$ $\mathtt{ACgcCGGTGGCTACGTCAAGGAGCCGGAGCGGGACTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCTTCAATCATAATCACCCACAACGTCTCGCCAGATAC$ ACCTCCCGACCTACACCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGGCTTGAGAG ACCCTCGGGAGGGACGGGAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAA ${\tt GCGCTTCTTGAGGGTCGTTAAGGAGGAGCCCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAGCGCTGTGAGAAGCTTGGCGTGAGCTTT$ GTGGCTGAAGCCGAAGGGGAAGAAGAAG// CAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACCCGCAGGACXXXCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGCGGTCATCGACATCTACGAGTACGACATACCC ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCCGTCATCAGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAGCCCTACTTCT ${ t TTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTCGACATCGAGACGCTCTACCACGAGGGAGAAGAGTTTGGAA$

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106/186

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GTT
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              CIT
                    GAA
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                   TTT
GGA
     ATA
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AGA
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CIT
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          GGA
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               ACA
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          GCC
TGA
               AAG
                    AAG
               AGC
      ATT
           GAT
                    CTA
           GAA
               GCA
                    ACT
      AGA
          648
               594
                    540
     702
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(PCNA) - JDF-3 fusion protein (Fig. 17-LL)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

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CIT
                          GTT
                                GAA
                                     AGA
                                           CTC
                                                ATG
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           TAT
                      GAA
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                                                           AGC
          GGA
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                                      CCA
                           486
                                432
                                      378
                      540
                                           324
                                                 270
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                594
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GAAGAAAAAGTTCCTCGGCAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACCCGCAGGACXXXCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGCGGTCA $\tt CCCTACTTCTACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGGT$ $\mathtt{AGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCCCGGCTCCAGCACCGGCAACCTCGTCGAGTGGTTC$ GCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGCTTGAGAGGGTCGCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACG GTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAAACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTTCGGCAA ${ t TCCTTACGTTGAGGTTGTCTCCACCGAGAAGGAGGATGATTAAGCGCTTCTTGAGGGTCGTTAAGGAGAAGGACCCGGACGTGCTGATAACATACAACGGCGACAACT$ GAGACGCTCTACCACGAGGGAGAAGAGTTTGGAACCGGGCCGATTCTGATGATAAGCTACGCCGATGAAAGCGAGGCGCGCGTGATAACCTGGAAGAAGATCGACCT $^\prime/$ ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCGTCATCAGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAG

Fig. 17 LL (cont)

 $\tt GTAGGAGCTACGACGTTGCCCCCGAGGTCGCTCACAAGTTCTGCAAGGACTTCCCCGGCTTCATTCCGAGCCTGCTCGGAAACCTGCTGGAGGAAAGGCAGAAGATP$ GGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATGCGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCG $\tt CCGCACGTAGCCATAGCGAAGCGTTTGGCCGCCAGAGGTGTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAG$ $\mathtt{CTGCCCGGCCTTCTCGAACTCGAATACGAGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGCAAGATAACCACGCC$ AAGTCCTCTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAA ${ t TGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTP$ ${ t G}$ GAGCGGGGACTGTGGGACATATCGTAGACTTTCGTAGTCTCTACCCTTCAATCATAATCACACAACGTCTCGCCAGATACGCTCAACCGCGAGGGGT GCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGGCTTGGCGCGTGGCTGA ${ t TTGTCAGGGAAGTCACCGAAAAGCTGAGCAAGTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGC$ CGGGCTTGAGATAGTCAGGCGCGACTGGAGCGAGATAGCGAAGGAGGACGCAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCGTCAGAA

Sac7d gene (ACCESSION No: M87569) (Fig. 17-MM)

TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAA TAA AAG N G K T G R G A V S E K D A P K E L AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA M V K V K F K Y K G E E K E V D T ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT K K AAG AAG GIT V W R V G K TGG AGA GTA GGC AAA M V A ATG GTG TCC F T Y D D
TTT ACC TAT GAC GAC S 67 201 54 162 36 108 18 54

Sac7d-Taq DNA polymerase fusion protein (Fig. 17-NN)

K I K K V W R V G K M V S F T Y D D AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC

N AAT G K T G R G A V S E K D A P K E L GGT AAG ACA GGT AGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA

L D M L A R A E R E K K //TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG //

G G G // GGC GGC GGT

V T S G M L P L F E P K G R V L L V GTC ACT AGT GGG ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTG GTG

GGC CAC CAC L A Y R T F H A L K G L T T TAC CGC ACC TTC CAC GCC CTG AAG GGC CTC ACC ACC

D GAC A GCC A GCC acc A SCC A 9 9 A GCC CIC CIG E GAG S S R G D Y GAC TAC CII I ATC I K AAG A GCG D GAC Y Q R H CGC CAC R L G G G T ACC TTT K AAG M ATG cgg R р Ф A GCG . d E GAG E GAG A GCC CIC CGG E GAG 900 P A GCC . GAG D G D A V I V V GAC GGG GAC GCG GTG ATC GTG GTC GAA K AAG CAA GTG GIC V A Y G G Y K A G R A P T GCC TAC GGG GGG TAC AAG GCG GGC CGC ACG W CII AAG CIG T ACG L T G D E S D CTG ACC GGG GAC GAG TCC GAC D GAC A GCG L W E K Y G CTT TGG GAA AAG TAC GGC S D R I H V
TCC GAC CGC ATC CAC GTC E GAG P CCG CIC Q CAG A L GCC CTC G Y GGC TAC R K AGG AAG A X Y G F A K S L L GCG OTC TAC GGC TTC GCC AAG AGC CTC CTC 9 9 9 CGG S CIG Y TAC I K E C ATC AAG GAG CIT E A GAG GCG E GAG K AAG W TGG D GAC D P CIG QTC GAC A GCC E GAG CGC R CIG D GAC CIG E GAG AAC AAC CIC I F D A K A A GCC ATC Ч CTT CCC QTC V GTG TGG R P D Q AGG CCC GAC CAG H P E G CIC AAG R CGG D L L GAC CTC CTG 999 1 L A T A ACC GCC × 999 999 S AGC R CGC K AAG QTC D CAC S AGC I TGG W Y CIG 9 9 9 CIC GAA AAG AAG AAA AAA

Fig. 17 NN (cont)

E GAG acc A မှ မှ Y TAC acc A CIC Ciè F. R AGG CTG L R E CTG AGG GAA GIG CIC acc A E GAG R D $_{\mathbb{R}^n}$ L K E A R G L L A K D L S V L AGG GAC CTG AAG GAG GCG CGG GGG CTT CTC GCC AAA GAC CTG AGC GTT CTG QTG CIG N AAC A GCC E GAA CCC CTG GAG GTG GAC TTC GCC AAA AGG CGG GAG CCC GAC CGG GAG AGG CTT A A A CTG GAC D V GAC GTG CIC E GAG TGG CIG ၁၅၅ ၁၅ TTT AGG E GAG T ACG TTT CIG TGG R BGG GCC GCC CCC P CCT E GAG G L G L P P G D D P M L L GGC CTT GGC CTC CCG CCC GGC GAC GAC CCC ATG CTC CTC V GTG AAG E GAG E GAG Y TAT E GAG S N T T P E G
TCC AAC ACC ACC CCC GAG GGG CIT R AGG GGG GGC CGG GTC CAC CGG GCC A GCC R AGG CIT S R K A GCG CIC S CTT CIG CIT R A G E GGG GAG TTC AGCT E GAG E GAG E GAG CGC R GTC မှ မရှိ E GAG LLL CIG TTG CIG GAG CGG R E GAG A GCC GGC AGC A GCC S A GCC A GCC 222 P E GAG DDD 4 CIG GCC A HCAC M ATG CIC R AGG TGG E GAG M ATG V A R R Y P E P Y K A CIT CIC 555 A W A D L TGG GCC GAT CTT CIC V A E E GTG GCC GAG GAG E A T G GAG GCC ACG GGG S P P CIT H E F CAC GAG TTC E R C GAG AGG TGG 922 P CTT E GAA CIC CIC Y DIO T AAC ATC QTG 999 9 GCC A TTC CTT CGG

17 NN (cont)

9 9 9 I ATC S AGC 9 9 9 I N AAC CGG S AAG CIC . ලේඋ ලේ Q I E CAG ATA GAG CGC K T E AAG ACG GAG S D P N L
TCC GAT CCC AAC CTC OTC R R A F I A E E G W L L V A L D Y CGC CGG GCC TTC ATC GCC GAG GAG GGG TGG CTA TTG GTG GCC CTG GAC TAT CIG CGC R E A L H 999 9 555 A F TTC aag E GAG Q CAG S AGC L R CTC AGG TGG W ACC CAC E GAG K T G K R S T S AAG ACC GGC AAG CGC TCC ACC AGC CIC T ACC E R V L F D E GAA AGG GTC CTC TTT GAC GAG ITA GGG A GCC CGC ටුටුට අ TAC Y Q CAG R D I CGG GAC ATC V L A H L S G D E N L I GTG CTG GCC CAC CTC TCC GGC GAC GAG AAC CTG ATC I D ATT GAC Q N I P V R T P L G Q R CAG AAC ATC CCC GTC CGC ACC CCG CTT GGG CAG AGG I V E 299 9 V GTG F N Q T A T K T BAAG ACC M S ATG TCG D P GAC CCC A GCC TTC F 222 đ K I L 3 AAG ATC CTG H T CAC ACG CTG I A GCC CIG TIG · GAG H M ATG р Ф CGC E GAG CGC R CGC R D GAC Y T ACC A GCC CIC CGG R CIC CAG A A V L E A GCC GCC GTC CTG GAG GCC L G L P A I CTA GGG CTT CCC GCC ATC T G R ACG GGC AGG I H P TTT S A GCG A GCC Y R E Q S CAG AGC S W AGC TGG R AGG Q E CAG GAG A GCC CGG R K AAG G Y GGG TAC TTC M ATG CIC CTA R AGG LCTA T ACC D d TTC AGT ACC GCC A I ATC ACC.

P K AAG org V E M G A R M L L Q V H D E GAA ATG GGG GCC AGG ATG CTC CTT CAG GTC CAC GAC GAG T A A D L M K L A M V K L F P R L ACC GCC GCC GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC TTC CCC AGG CTG E T GAG ACC K E R A E AAA GAG AGG GCG GAG S V R E A A E R M A F N M P V Q AGC GTG CGG GAG GCC GAG CGC ATG GCC TTC AAC ATG CCC GTC CAG CIC L TTC 9 9 9 A V A R L A K GCC GTG GCC AAG R R R Y V P D CCA GAC CIG GTC CTC E V M E GAG GTC ATG GAG L E A
CTA GAG GCC E A CGG R 999 9 E GAG မ မ မ

H H *
CAT CAT TAA CIC ote V Y P L A V TAT CCC CTG GCC GTG S A K E G I D G R G G G TCC GCC AAG GAG GGC ATT GAT GGC CGC GGC GGA GGC P L E V E V G CCC CTG GAG GTG GGG 999 9 I ATA . GGG H H H CAT CAT CAT E D W GAG GAC TGG H

Taq DNA polymerase-Sac7d fusion protein (Fig. 17-00)

// GGC GGC GGT

D GAC GTC T S G ACT AGT GGG GGC CAC CAC CIG M ATG GCC A CIG TAC DDD P R T F H A CGC ACC TTC CAC GCC CIC F E P K
TTT GAG CCC AAG CIG မရိုင် ရ K G BAAG GGC CGG R GTC V L T CIC CTG ACC V GTG

Fig. 1700 (cont)

A GCC 9 9 9 A GCC CIC D GAC A GCC CIG E GAG S A GCC S I ATC CTT I D GAC AAG a GCG D GAC TTC CGG R ACC Y TAC 999 9 TTT CGC AAG CGC CCG P Q CAG CGG R E GAG A GCG CIC 232 P H E A A GCC E K GAA AAG acc A CIC D G D A V I V GAC GGG GAC GCG GTG ATC GTG р Р K AAG GAG' CGG TGG V Q A X Y G F A K S L
GTG CAG GCG OTC TAC GGC TTC GCC AAG AGC CTC CIG CIT CAA T ACG GTC U A GCG T ACC CTT S Y E GAG CIC AAG D GAC P P D GAC କ୍ କ୍ରକ୍ତ 999 9 TGG W ၁၅၃ ၁ A GCC CIC L CGG R R AGG ၁၉ ၁ Þ R I CGC ATC -ଜ୍ୱେଜ -GAC GAA CIC AAG Y TAC SS CIG Y \asymp K AAG TGG CTT E GAG E GAG E GAG I ATC Y TAC Ħ Y TAC H 222 d CIG S GTC K AAG A GCG K AAG × GTC R I D GAC 9 9 9 A GCG GIC CIG E GAG GAC E GAG Ш A GCC CIC E GAG N AAC CIG ၁၅၅ ၁ I D GAC CTG CTT CAC CGG GAC AAG CGG R W TGG R AGG CIC GTC OTG U ole A E GAG 9 9 9 ეეე ტ DDD 4 222 P T ACC A GCC D GAC CIG Ø E GAG G G G D GAC GCC A CIC 222 A A GCC CIG GTC CAG G G G D GAC S AGC CIG T ACG D GAC CIG GAA K AAG Y TGG AAA K K AAG CIG CC_A

Fig. 17 00 (cont)

A GCC 9 9 YAC A. GCC CIG CIG GCC A E GAG CIC TTC AGG N AAC E GAG CGC R QTG CIC CIG R AGG A GCC GTG E GAA A GCC W TGG D GAC A GCC AGC CIG CIG R AGG CIG P K A TGG T ACG E GAA A R G G R V GCC AGG GGG GGC CGG GTC R AGĠ D GAC OIC T F V L S R K E P M W TIT GIG CIT TCC CGC AAG GAG CCC AIG IGG CIG T GAG E E A GAG GAG GCG G R GGG AGG K'L E A AAG GAG GCG A. GCC 9 9 9 222 A TOO P E GAG GTG GAC TTC GCC AAA AGG CIT E GAG S CTT R AGG CIT QTC CIC S N AAC ၁၅၅ ၁ L E E A CTT E G 999 9 CGG R TTC R AGG A GCT T ACC CIC E GAG E GAG CGC R GTC T ACC P CCG 9 9 9 TTT e Gag R CGG CAC CIG TIG) D 222 Å CIT 9 9 9 A A GCC GCC E G GAG GGG R A P W CTC GCC E GAG A GCC A GCC 9 9 S AGC S CGG R AGG 999 9 HCAC D GAC CIC CIG GAG CCC GAC CGG GAG 522 232 £ H gag Gag M ATG CIC CTT OTG V P E P D GAC AAA A CIC DDD P GIG E GAG CIT TCC s A GCC HCAC D GAC A D L GCC GAT CTT 222 4 TTC A GCC E GAG CCG P A GCC R CGG CIG E GAG M ATG R AGG T ACG CGC R Y Y E GAA CIC S AGC JIC F CIC G G G G CIC Y TAC Y TAC _ GGG CIC GTT 9 9 9 . GCC GCC A cee R TTC မှ မှ မှ I ATC A GCC CIG CH CIT

FIS. 1700 (cont)

S AGC မှ မှ AAG CIC CGG ATC I ATC CGC R CIG GIC CGC R TCC S CGC R V GTG TTC GTC AAG CGG R D P N L Q N I GAT CCC AAC CTC CAG AAC ATC L H T R F N Q T A T A T CTC CAC ACC CGC TTC AAC CAG ACG GCC ACG GCC ACG E GAG JIC F I ATA S T Y I D P AGC ACC TAC ATT GAC CCC A GCC R CGG CAG GAG A GCC E · A GAG GCC L Y CTC TAC F I CAC CTG GAA E GAG CIC K AAG I 222 A မှ မှ မှ R AGG T ACC GTG GCC 9 9 AGG CGG R OTG I ATC 999 9 GAC M ATG GAG D GAC CTG ole V K AAG GIC TCG CCC P GAG ACC I A GCC E GAG CIC CGC R A GCC ეეე ტ TIG CIG CIG CAC CAC **G**GG K AAG S TTT V R T E GAG E GAG H M ATG T ACG CIC P D L I H I ATC W TGG T ACC E GAG CGC R E GAG CGC # CTG S LCTA s AGC 9 9 9 CIC CGG T ACC 9 9 9 TTG A GCC Q CAG AGG S A GCG A GCC D GAC OTG 522 A 9 9 9 Y TAC AGG GCC A -------A GCC CIT S AGC E GAG CGG R R L S AGG CTA AGT CGG R K AAG W TGG CTG ය යෙ D D AAC E GAG R AGG T ACC M ATG CIG . GAC cag T ACG Y A GCC I TTC ATC Y TAT R AGG

Fig. 1700 (cont)

K S V AAG AGC GTG P GAA ACC V E T GTG GAG ACC A A M ATG K E R A E A V A R L A K E V AAA GAG AGG GCC GAG GCC GTG GCC CGG CTG GCC AAG GAG GTC **G**GG GCC A D L M K L A M V K L GAC CTC ATG AAG CTG GCT ATG GTG AAG CTC L F G R R R Y V P D L E A CTC TTC GGC CGC CGC TAC GTG CCA GAC CTA GAG GCC R E A A E R M A F N M P V CGG GAG GCG GCC GAG CGC ATG GCC TTC AAC ATG CCC GTC R M AGG ATG L L Q V H D E CTC CTT CAG GTC CAC GAC GAG T V F P M ATG CTC R AGG R V E GAG Q CAG E GAG CIG A GCC E GAG မှ မ 9 9

CIC OIG A S Y A GCC 222 P K E G I D G AAG GAG GGC ATT GAT GGC CIG A V GCC GTG 333 d CIG E GAG GTG CGC R E V G GAG GTG GGG 9 9 GGA GGC GGG CAT CAT CAT I ATA ය අ E GAG D GAC CAT TGG W

CAT CAT ==

M ATG K AAG I ATA QTG AAG AAG V K F K Y K G GTA AAG TTC AAG TAT AAG GGT T ACA aag GGT G V W R V G K GTT TGG AGA GTA GGC AAA R G A AGA GGA GCT V S M V S E E K E V GAA GAG AAA GAA GTA E GAG K D A 3 AAA GAT GCT TTT P K E T ACC Y DAC TTA D GAC STCA

A GCA

R AGA

E GAG

K K // B AAG AAA //

TAG

Pfu DNA Polymerase (WT) -Sac7d fusion protein (Fig. 17-PP)

ttacataact gaagaaggaa acctgttat taggctattc aaaaaagaga acggaaaatt gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga caactctaca cctcccctat tttctctctt atgagatttt taagtatagt tatagagaag ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat aataccaatg gagggggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tgatgtagag aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta aaagattgaa gaagttaaga aaataacggg ggaaaggcat ggaaagattg tgagaattgt taagatagag catgatagaa cttttagacc atacatttac getettetea gggatgatte tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga agttacttat aatggagact cattcgactt cccatattta gcgaaaaaggg cagaaaaact tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct

118/186-

Fig 17 PP (cont)

gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag gaaggagete gaagaaaagt ttggatttaa agteetetae attgacaetg atggteteta ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac atacataaat tcaaagctcc ctggactgct agagcttgaa tatgaagggt tttataagag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc tagageceta tatecetega ttataattae eeacaatgtt teteeegata etetaaatet

)

aaagtataat ttcaagctcc ttcttcttca tgacagatga aaaattgttt tgtctctttt tttgctccaa gcagagccgc tccaatggat aacacccctg ttcccgcacc caagtccgct tcaagatttt ctaaaagaat tttaacggcc tcctcgtcaa tttcgacgac gtagatcttt cagagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatggt tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta aacttcctgg cttaacatta aaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga taacttttac agaaataact gtctcaaatt atgacaactc ttgacatttt tacttcatta ttccgggagt tttgtattgc tctagccaag gtttgggatt tttgaatcct ttaactctgg acaatttttt ccttgtatct cctaatgtat aagcaagcca aaggagagta gatgctacct ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag aggcgatggt ccaattagca atagggcaat tctagctgag gaatacgatc ccaaaaagca

ccagggtaat gtttttaagt atgaaatttt tctttcatag aggaggnnnn nngtcctctc ctcgatttcc ttggttgtgc tccatatgat aagcttccaa agtgggtgtt cagactttta gacactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg gttgagaaaa gtagaagcgg cactactcag atgcttcccc aggaatgagg ttgttgtagc tcntcccnga aagattgaga tgttcttgg //

 $^\prime/$ ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TCA AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG AAA //TGA

Sac7d - Pfu DNA Polymerase (WT) fusion protein (Fig. 17-QQ)

gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga taagatagag catgatagaa cttttagacc atacatttac gctcttctca gggatgattc ttacataact gaagaaggaa aacctgttat taggctattc aaaaaagaga acggaaaatt caactctaca cctcccctat tttctctctt atgagatttt taagtatagt tatagagaag ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc // ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAA GAA GAA GTA GAC ACT TCA AAG ATA AAG AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat GGT AAG ATG TTA GCA AGA GCA GAA AGA ACA GGT AGA GGA GCT GTA GAG AAG AAA // AGC GAG AAA GAT GCT CCA AAA GAA TTA

,

Fig. 17 QQ (cont)

aaagattgaa gaagttaaga aaataacggg ggaaaggcat ggaaagattg tgagaattgt gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc aataccaatg gagggggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta tttggaacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tgatgtagag aaggttgaga aaaagtttct cggcaagcct attaccgtgt ggaaacttta gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag tcacgaagga gaagagtttg gaaaaggccc aattataatg attagttatg cagatgaaaa tgtggacatc ttcgaatacg atattccatt tgcaaagaga tacctcatcg acaaaggcct aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag tagageceta tatecetega ttataattae eeacaatgtt teteeegata etetaaatet

tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac atacataaat tcaaagctcc^{:k}ctggactgct agagcttgaa tatgaagggt tttataagag gaaggagctc gaagaaaagt ttggatttaa agtcctctac attgacactg atggtctcta gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta aacttcctgg cttaacatta aaaaatccta gaaaagcgat agatatcaac ttttattctt caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt aggcgatggt ccaattagca atagggcaat tctagctgag gaatacgatc ccaaaaagca actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac

Sac7d - PFU DNA POLYMERASE (V93 R OR E) fusion protein (Fig. 17-RR)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)
V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

AAG ATA AAG AAG GTT TGG ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC TCA

GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GCAAAAGCAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA GGAGACTCAT GTTGAGGTTG GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // TAAAAGAAGT 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG GAGTTTGGAA TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA AGTGGAGAGA CCAACATACA ATGCAGAGAA CTCATCGACA AAGATTGTGA //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC TCGCATTCCC TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG TACATCGAGT TAGTATGGAA GGAGCTCGAA TTAAGACAAA AATGAAGGAA ACTCAAGATC 2328 3 6 0 720 540

PFU DNA POLYMERASE (V93 R OR E) -Sac7d fusion protein (Fig. 17-SS)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA

GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG GGATACATAG ATAAGGCGAT ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG 1680 GCTCTAGAAT TIGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA ATGCAGAGAA GGAGACTCAT GTTGAGGTTG CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC $^{\prime\prime}$ atg gtg aag gta aag ttc aag tat aag ggt gaa gag aaa gaa gta gac act tca TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 CACTAGAGGC TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC TCGCATTCCC AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 TATCAAGCGA AAGGCCCAAT GAGAGAGATG ATAAAGAGAT TTCTCAGGGAT TATCAGGGAG 600 TATAATGATT TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC ACCATTGGAA GAGATGGAAG CGAGCCCAAG AAGGATCCTG ACATTATAGT TACTTATAAT GATATAGAAA CCCTCTATCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG TCTACGGATA TTATGGCTAT 1500 2328 AAGACCTCAG ATACCAAAAG CGAAGGAGAA 540

PFU DNA POLYMERASE (G387P/V93R OR E)-Sac7d fusion protein (Fig. 17-TT)

AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA

TTA GCA AGA GCA GAA AGA GAG AAG AAA //

ATG

AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC

TAT GAC GAC

TTA

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T)

GATAGAACTT ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA

GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAAT AAAGAAAAAG CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT GTTGAGGTTG CTCATCGACA 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT ${f N}$ GGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC TCGCATTCCC TATCAAGCGA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT ATTACTTGGA AAAACATAGA TCTTCCATAC GATATAGAAA CCCTCTATCA CGAAGGAGAA TGAAGAAGCT 1920 GTGAGAATAG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG TCTACGGATA TTATGGCTAT 1500 1140 CTCAGGGAGA GCTACACACC 2328 720 540

// ATG GTG AAG AAG AAG AAG GTA TTA ACA GIT AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC GCA GGT AGA GGA GCT TGG AGA GTA GAA 999 AGC GAG AAA GAG ATG AAG AAA GAT GCT GIG ICC TTT ACC CCA AAA GAA TTA TAT GAC GAC ACT TCA

PFU DNA POLYMERASE (G387P/V93R OR E)-Sac7d fusion protein (Fig. 17-UU)

FIG. 17UU (cont)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ${f N}$ GGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACACC ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GTTGAGGTTG GAGTTTGGAA CAAGATXXXC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T) 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC 720 360

AAG AAG GTA AAG TTC AAG TAT AAG ACA AAG TTA GCA AGA GCA GAA AGA GIT GGT AGA GGA GCT GTA TGG AGA GTA GGC GAG AGC GGT GAA AAA ATG GAG AAG AAA // GTG TCC TTT GAG AAA GAA AAA GAT GCT GTA GAC ACT AAA GAA TAT GAC GAC TCA TTA

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T)

// ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAA // AAA GAA GAC ACT TTA TCA

GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GATAGAACTT TTAGACCATA#CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA 180 GCTACACAGG. TGGATTCGTT AAAGAGCCAG AAAAGGGGGTT GTGGGAAAAC 1200 ATAGTATACC AGTGGAGAGA ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GAGTTTGGAA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 GAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA CCAACATACA CACTAGAGGC GTTGAGGTTG AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT 1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA //ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GCNATAGCNA CCCTCTATCA CGAAGGAGAA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA CTCGCAATAT ATGAGCAGAT AACAAGACCA ACGGAGATGT TCATTACTCG TGGTTTAGAG ATAGTTAGGA TCGCTCCTCA AGTAGGCCAC TAGATTTTAG AGCCCTATAT CCCTCGATTA TTCCATTTGC TGAAGAAGCT TCTTCCATAC AAAGAGATAC

TGA

KOD DNA POLYMERASE - Sac7d fusion protein (Fig. 17-WW)

GTTGACGTCG GAGCTTGGGA ACCGGCGAGA ACCTTGAGAG GCCGTCTTCG TATCCTGTGA ATTCAGAGGA AAGCGCTGTG AAAGACCCGG AGCTACGCCG GACATTGAAA CTCTCTACCA CGAGAGCATC ACGGTTGTAA CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CAGAAGATAA GATACGCTCA GTGTACCTAG CGGCAGAGCT GCCTATGAGA CTCTGGGACG CTCATAGACA AGGGATTAGT GCCAATGGAA GGCGACGAGG GAGGTCTGGA AAGGAAAACG TACAGGCAGA AGGGCGCGCT TTCTGCAAGG AGGAGTTCCT GTCAGCCGAA TCTCGACGGA ACGAGGAAGG CAGCAGTTAT TGACATCTAC GAGTACGACA AACTCTACTT" TACTCATCCG CGGTTAAGCG CGGTTGAAAAG AGAAGAAGAT ATTTTAGATO ATGAAGGAGG GGAATGAGCT TAAGACGGAC TGGGCGACAG AAAAGCTCGG ACGTTCTCAT GCGAGTTTAA GATTGAGTAC GGGCCATCAA ACTTCCCAGG TCTCCCGCTC CCATCAAGGA GGTACTGCAA ACAGAGAAGG GGAGTGTGCA GAGAGCGTAA GATCCTGGCA AACAGCTACT GAAGGCCACG ATTGACCCGA ATTTATCCCG GGCCCCGAAC AAGCCCGATG CAGCACTGGC TCCGATGGAG GCCCAGCTTT AGTCGCCCGC GGAGAAGGTT TACGCTGAGG GATAAACCTG GTTTGCCGTC AATAAACTTC AACCTACAAC GAGGGAGATG ATAAAGCGCT GGCCAGGGTG ATAACTTGGA TGAGGGCGAG GAGTTCGCCG GATAGAGGAA AAGTACGGCT ATGCAAGGAA CCTGTACCCC CTATGTAAAA GAGCCCGAGA AACCTCGTTG TACTCGATGG GGCGACAACT CAGGACXXXC GACCGGACTT AGCCTGCTTG TATGACGTTG TCAATCATCA CCCACATACA GAAGTGAAGG GCCCTCGGAA GTTCAGAAGA CGGCCTGGGG ACGGTTACTA AAAAGGAGCT TCCTCCGTGT AGGGGCCAAT TCGAGAGGAA GAGACCTCCT GAGGGTTGTG AGTGGTTCCT CTCGCTTAAT AAGATGCGAA AAATAACCAC CGCTTGAGGC GACGGATACA GGGATGGAAG TCGACTTCGC AGAACGTGGA TACCCTTCGC TAACCGCCGA GAGGCACGGG TTGAACCCTA CTTCTACGCC TTAAGGTAAT CCCCACAGGT TCACCCACAA AGCTGAAAAT CAGCGATAAG AGTTCCTCGG CTGTCATAAG AATTTTCAAG CGT (ALL POSSIBLE CODONS FOR ARGININE) GLUTAMIC ACID) CCTTATGATA CGGCTATGCA GCTCCTCGAT AGAGGAGAGG CGTCTCGCCG CGGCCAGTCC GGTCACATAC CGTTTATGAA CGAGCCGAAG TCTCCCCTAC GCTCGCCTTC CAAGCGCTAC GAGACCAGTT CTACAGCGAC AAGGGAGTAC CGGCCACCGC GGAGAACATA GGCCAGAAGA CCTCAGGAAG AGCCTGGGAA CTTCGATCTC TGTGAAGGAG GGACAAGATA CTATCTGAAA 960 840 600 360 1440 1380 1080 1020 900 780 720 660 480 1260 1200 1140 540 420

ACCGACGGAT

TTTTTGCCAC AATACCTGGA GCCGATGCTG AAACCGTCAA AAAGAAGGCT

GACCCGACGA GTGATCCACG AGGATAGTCA GAGACGCAGG ATGGAGTTCC TCAAGTATAT CAACGCCAAA CTTCCGGGCG CGCTTGAGCT CGAGTACGAG AGACAGGTTG GTTTGAGTGC TTGGCTGAAG CCGAAGGGAA CT 2325 GTTGAGAGAA TACATCGTGC GCCGTTGCCA // ATG GTG AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG AAA // AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TCAAGGGCTC TGGGAGGATA GGCGACAGGG CGATACCGTT AGCAGATAAC AAGAAGTTAC CGAGGGTTCT TGAAGCTTTG CTAAAGGACG GTGACGTCGA GAAGGCCGTG 1920 CAACGCGCGG AACGCGGCTT TTCTGAGAGC CTTCGGTTAC CGCAAGGAAG ACCTGCGCTA CCAGAAGACG 2280 AGCACAAGTA CGACGCCGAG TACTACATTG AGAACCAGGT TCTCCCAGCC 2220 AGAGGTTGGC GAGGGATTTA AAGGACTACA AGGCAACCGG TCCCCACGTT 2040 CGCGAGAGGA GTCAAAATAC CGAAAAGCTG AGCAAGTACG AGGTTCCGCC ACTIGAGATT GTGAGGCGTG CTTCGTCACG AAGAAGAAGT ATGCGGTGAT AGACGAGGAA GCCCTGGAAC ACTGGAGCGA GATAGCGAAA 1860 CGACGAGTTC 2160 GGTGATAAGC GGAGAAGCTG 1980 2100 TTA

Sac7d - KOD DNA POLYMERASE fusion protein (Fig. 17-XX)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

// ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TCA AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG AAA // AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC

GACATTGAAA CTCTCTACCA TGAGGGCGAG GAGTTCGCCG AGGGGCCAAT CCTTATGATA CGAGAGCATC CAGCAGTTAT TGACATCTAC GAGTACGACA TACCCTTCGC CAAGCGCTAC 360 GAGGTCTGGA AACTCTACTT TACTCATCCG CAGGACXXXC CAGCGATAAG GGACAAGATA 300 CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA TAACCGCCGA GAGGCACGGG 180 GTTGACGTCG TCTCGACGGA GAGGGAGATG ATAAAGCGCT TCCTCCGTGT AGCTACGCCG ACGAGGAAGG GGCCAGGGTG ATAACTTGGA AGAACGTGGA CTCATAGACA AGGGATTAGT GCCAATGGAA GGCGACGAGG AGCTGAAAAT GCTCGCCTTC ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA AGTTCCTCGG GAGACCAGTT 240 AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT TTGAACCCTA CTTCTACGCC 120 //ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC CTGTCATAAG AATTTTCAAG 60 TGTGAAGGAG TCTCCCCTAC

GCCGTTGCCA GTGATCCACG GGCAAGATAA GGCTTCTACA ATGGAGTTCC ACCGACGGAT ATAACGATGA AGGGCGCGCT TACAGGCAGA CAGAAGATAA GATACGCTCA GTGTACCTAG CGGCAGAGCT GCCTATGAGA CTCTGGGACG TCTCCCGCTC GAGCTTGGGA GCCGTCTTCG AAAGACCCGG ACGTTCTCAT AACCTACAAC GGCGACAACT TCGACTTCGC GTTGAGAGAA GACCCGACGA TACATCGTGC AGGATAGTCA GAGACGCAGG TTCTGCAAGG ACCGGCGAGA AGACAGGTTG GTTTGAGTGC TTGGCTGAAG CCGAAGGGAA CT //TAG 2325 TCAAGGGCTC TGGGAGGATA GGCGACAGGG CGATACCGTT CGACGAGTTC AGCAGATAAC AAGAAGTTAC CGAGGGTTCT TGAAGCTTTG CTAAAGGACG CCATCAAGGA GATAGAGGAA AAGTACGGCT TTAAGGTAAT CTACAGCGAC GGTACTGCAA GGAGTGTGCA GAGAGCGTAA GGGCCATCAA GATCCTGGCA AACAGCTACT ACGGTTACTA AGAAGAAGAT GAAGGCCACG ATTGACCCGA ACTICCCAGG ATTIATCCCG ATGAAGGAGG GGAATGAGCT AGGAGTTCCT TCCGATGGAG ACCTTGAGAG AGTCGCCCGC TACTCGATGG AAGATGCGAA GTCAGCCGAA GGAGAAGGTT AAAAGCTCGG AGCACAAGTA AGAGGTTGGC CAACGCGCGG ACTTGAGATT TCAAGTATAT ACAGAGAAGG ATGCAAGGAA ATTTTAGATO TAAGACGGAC GATAAACCTG TGGGCGACAG TTCTGAGAGC AACGCGGCTT TTTTTGCCAC CGACGCCGAG GAGGGATTTA CGAAAAGCTG AGCAAGTACG CAACGCCAAA CTTCCGGGCG CTATGTAAAA GAGCCCGAGA GAGGGTTGTG GGAGAACATA GGCCCCGAAC AAGCCCGATG AAAAGGAGCT AATAAACTTC CTTCGGTTAC CGCGAGAGGA GTCAAAATAC CTTCGTCACG AATACCTGGA GCCGATGCTG CCTGTACCCC CAGCACTGGC AACCTCGTTG GTTTGCCGTC TACTACATTG AAGGACTACA AGGCAACCGG TCCCCACGTT GCCCAGCTTT TACGCTGAGG GTGAGGCGTG TATGACGTTG AAGAAGAAGT AGCCTGCTTG CCCACATACA CGCAAGGAAG ACCTGCGCTA CCAGAAGACG TCAATCATCA GCCCTCGGAA GAAGTGAAGG AGAACCAGGT TCTCCCAGCC GCCCTGGAAC GGTGATAAGC AGGTTCCGCC GGAGAAGCTG GTGACGTCGA GAAGGCCGTG ACTGGAGCGA ATGCGGTGAT AGACGAGGAA CGCTTGAGCT CGAGTACGAG AAACCGTCAA AAAGAAGGCT CGGCCTGGGG TCGAGAGGAA GCTCCTCGAT GAGACCTCCT CCCCACAGGT TCACCCACAA AGTGGTTCCT CTCGCTTAAT AAATAACCAC GACGGATACA GGGATGGAAG CGCTTGAGGC GATAGCGAAA CGGCTATGCA GGTCACATAC CTATCTGAAA AAGGGAGTAC AGAGGAGAGG CGGCCACCGC CGTCTCGCCG GGCCAGAAGA CCTCAGGAAG CGGCCAGTCC AGCCTGGGAA CGTTTATGAA CTTCGATCTC CGAGCCGAAG 1140 1080 2040 1980 1860 1800 1740 1680 1500 1440 1200 2100 1620 1560 1380 1320 1260 1020

Sac7d-Vent DNA POLYMERASE FUSION PROTEIN (Fig. 17-YY)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

 $^{\prime}/$ ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TC $^{\mu}$

FIG 17. YY (cont)

GAC

AAG

AIG

AAG ACA TTA

GCA AGA GCA GAA AGA GAG AAA //

GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA

ATA

AAG

GAAAATATCA GCAATGAGGC CGGCGCTTAA AGGGTGGCAT CAAAGTGTAT GATCTTTTCC CCCAAGATTC AAACGGGCAG ATAGCTAAGG GATGAAGAGG GAGTATGAGG AAGAAAGCCA AGACACTACA GGGTATCCTA ATGCTCGATT GGATATAGGT GTATCCCCAG ACGTATGAGC TGGGAAACAG TATGAAGCAG AAAGACCCCG GTCGATGTTG AGTTATGCCG GATATTGAAA CTCATAGACA AGGGAACATC GAAGTCTGGA AAAACTGTGA CTTCTCAAAG AAAGAGAACG ATGATACTGG TATGCGGACA GAACAACTTA CTGACGGCTT TAGAGATGAC AGGCAAGATG ATAGGCAAAG ACGCGAGGAA GGGACGTCTC TCGGGAAGGA AAGAAAGCAT AAAAGCTGGG ATGTGATAAT AACTTACAAT TGTCCAATGA AAGAGAAATG CGTTTTATCA CAGCTGTGGT GAGTGCTCGA ACACTGATTA CATAACAAAA GATGGCAAGC TTATCCATGA AAGTTGTTAG AGACTCAGGC GCAGGATAAC GCTTTTACTT AGGAATTCCT AAGATATAAA TCTGCAAGGA ATACCCTTGA TTTATTTGGA TTTTAGGAAA CAGTTGTGCG AGAGGATGGG ATGAAGAAGA AGGGCTTGAT AGCTCATTTT ATGACTCCGC GGGAGTTTAA GCAGATTACC GAGAGGATTC AAACTACATA CTTTCCGGGC AAAAGAGGGC CCTGGGAGGA TGAACTTGCA GAGATCAAGC ATTCTTCCCC GAAAAAACTA GGCCAGAGTA AGATGTTGTA AACAAGGGGC GTACTCGAAG GGCTATTAAA GAAGAAAATG TTTCCGCAGT AACCAAAAGC AGTTCGGCTT AAAGGTTTTA TTATGCCACA GATAAGAGAA AAGGACGATA TGATAGTTTT TGAGGGAGAT TCCCATGGAG TGACATTTAC CGAGCATCCC AATAGAACTT TGCAGTGAAA TATTGAGGAG ATACCCGGGG AGGGATTTAA TTGGAAGTAG AACTCCAAAC ATAGAGGAAA GAATGTGCTG AAATCCACAA TGTAAGAATT CTGTACCCTT TATGTAAAAG CCGAACAAAC ACCGGCAACC ATGGAAGCTG GCCCAGTACT AAATTAGGAG GCTGTGGAAA GTCTTAGGAA GGGGACAATT ATAAAGCGTT GAATTTGGAA GTCAGGAAAA GACCCTCATT GAGAAAATAG GAGGCTATAC TTTGTTACAA TIGCTIGCAA TTTATTCCCT AACCTCCCAA ATCACATGGA GGAGACGAGG GAATATGACA CAAGACXXXC ATAAAGGCAA ACGATGTTGC AGGACTACAA CAAAATACAG TAAGGAGAGA AAAAGCGCTA AAAAGCCTGA AGTTCGGCTT TTGACCCGAT CCATACTCGG CAATAATAGT TTAAAGAGGG TTCCAGGTCT AAAGCGTTAC ACAGCTATTA AGCCAGAAAA CTGATGAGGA AGCTGGCAAA CAATGGAAGA CAGAGGAAAT TCAAGGGTAG GGGACAAAGA TTGATTTGCC AAAATATCGA AGGGCGAGAT TAAAGGGCGA TCGTGGAGTG CGTATACGCT TTGTTCAAGT AGCTTAAGCT TACCCTTTGC CAGCTATGCG AATTTTTGGG TTCAGCCCTA CTATAATCCG GCTTGAGCTT AGCCATTGGC GGTTCCACTT ACTCATTAAA TAAGGTTCTT CGAAAAGAAA TACTCACAAC AGGTTTGTGG AAGTGTTGAA TTGGAGTGAG TGCAGTCATA GGACTTAATT AGAGTATAAA GCTGATAGGT CGCATGGGGG CGGCTATATG TCCGATAGTA GTATCTTTTA TGCCGCTATA TGAGGCAGTT AATCCACTTT ACATCCCGAA GTATCTCATA TTTGCCGTAT TGCTAGGGCA TGTTAAAGAA AATAATGATT CCTTGCCTTT CAAGCGTTAT GGGCAAAATA AAGGGAAGTT GAGACATGGA TATATATGCT AATTTTTAAG 1860 1800 1740 1680 1620 1500 1440 1380 1320 960 900 840 1560 1260 1200 1140 1020 720 660 600 780 480 360 300 240 1080 420

CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GGTAG 2325 ACAGAATACG ATCCTAGAAA ACACAAGTAC GATCCGGACT ACTACATAGA AAACCAAGTT ATAATAAGCT CCTCATGTCG TTGCCGGCAG CGATAGCAAA AAGACTTGCC GCAAGAGGGA TAAAAGTGAA ACCGGGCACA TACTTAGGAT ACTCGAAGCG ATATCGTTCT CAAAGGGAGC TTTGGATACA GAAAGGAGGA GGAAAGATAA GCGATAGGGT AATTTTACTT TTTAAGGTAT 2280 2220 2160 2100

Vent DNA POLYMERASE - Sac7d FUSION PROTEIN (Fig. 17-ZZ)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

GAAAATATCA GATCTTTTCC **AAACGGGCAG** GTCGATGTTG GATATTGAAA AGGGAACATC GAAGTCTGGA CTTCTCAAAG AAAGAGAACG GCAATGAGGC GTATCCCCAG CGGCGCTTAA AGGGTGGCAT CAAAGTGTAT ACGTATGAGC TATGAAGCAG CCCAAGATTC AAAGACCCCG AGTTATGCCG CTCATAGACA AAAACTGTGA ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CTATAATCCG AATTTTTAAG AGACACTACA GGGTATCCTA ATGCTCGATT TGGGAAACAG AAAAGCTGGG GGGACGTCTC CGTTTTATCA ATGACTCCGC; TATTGAGGAG ATAAAGGCAA TAAAGGGCGA GAACAACTTA AGCTCATTTT AGGCAAGATG AAGATATAAA TCTGCAAGGA ATACCCTTGA TTTATTTGGA ACGCGAGGAA TCGGGAAGGA AAGAAAGCAT TTTTAGGAAA CAGTTGTGCG AGAGGATGGG ATGTGATAAT TGTCCAATGA ATGAAGAAGA AGGGCTTGAT CAGCTGTGGT GAGTGCTCGA TGCAGTGAAA GGGAGTTTAA AATAGAACTT TAGAGATGAC ATAGGCAAAG GAAGAAAATG CCTGGGAGGA GAGATCAAGC ATTCTTCCCC GAAAAAACTA AGTTCGGCTT GTCTTAGGAA GGGACAAAGA AACTTACAAT GGGGACAATT TTGATTTGCC AAGAGAAATG ATAAAGCGTT TTGTTCAAGT GGCCAGAGTA ATCACATGGA TGAGGGAGAT GAATTTGGAA TCCCATGGAG TGACATTTAC CGAGCATCCC GATAAGAGAA ATAGAGGAAA GGCTATTAAA CTTTCCGGGC AAAAGAGGGC TTTCCGCAGT TGAACTTGCA CCGAACAAAC AACCAAAAGC TGATAGTTTT GCTGTGGAAA TCAAGGGTAG GTACTCGAAG GAATGTGCTG AAGGACGATA GTCAGGAAAA TTGCTTGCAA AAATCCACAA TTTATTCCCT TGTAAGAATT CIGIACCCII TATGTAAAAG ACCGGCAACC ATGGAAGCTG GCCCAGTACT AAATTAGGAG AACCTCCCAA GGAGACGAGG GAATATGACA CAAGACXXXC CAGCTATGCG GACCCTCATT TTCAGCCCTA TATATATGCT CCATACTCGG ACGATGTTGC CAATAATAGT AGCCAGAAAA CAATGGAAGA CGTATACGCT AAAATATCGA AGGGCGAGAT AAAGCGTTAC ACAGCTATTA TTGACCCGAT CTGATGAGGA AGCTGGCAAA AGCTTAAGCT AATTTTTGGG AGTTCGGCTT TCGTGGAGTG CAGAGGAAAT TACCCTTTGC CGGCTATATG CGAAAAGAAA GGACTTAATT TCCGATAGTA TACTCACAAC AGGITTGTGG AGAGTATAAA GTATCTTTTA GCTGATAGGT TGCTAGGGCA TGCCGCTATA TGAGGCAGTT AATCCACTTT ACATCCCGAA GTATCTCATA TGTTAAAGAA TTTGCCGTAT AATAATGATT CCTTGCCTTT CAAGCGTTAT GGGCAAAATA AAGGGAAGTT GAGACATGGA TAAGGTTCTT CGCATGGGGG 1380 780 540 1440 1320 1260 1200 1140 1080 840 720 660 600 480 1020 900 360 180 300

CAAAGCTCAA ACAGAATACG CCTCATGTCG GAAAAGCTTG AAAGCTGTAG AAGTTGTTAG AGATGTTGTA ATAGCTAAGG GATGAAGAGG GAGTATGAGG AAGAAAGCCA TATGCGGACA CTGACGGCTT TTATGCCACA ATACCCGGGG AAAAGCCTGA ACTCATTAAA TTGCCGGCAG ATAATAAGCT AACAAACCGG CTTAGATGCA TGGCTCAAGA ATCCTAGAAA ACACAAGTAC GATCCGGACT ACTACATAGA AAACCAAGTT ATATCGTTCT CAAAGGGAGC CGATAGCAAA AAGACTTGCC GCAAGAGGGA TAAAAGTGAA TTATCCATGA GCAGATTACC AGGGATTTAA AGGACTACAA AGACTCAGGC GCAGGATAAC GCTTTTACTT AGGAATTCCT TACTTAGGAT ACTCGAAGCG TTTGGATACA GAGAGGATTC AAACTACATA AACTCCAAAC AAAGGTTTTA GAGGCTATAC AACAAGGGGC GGAAAGATAA GCGATAGGGT GAGAAAATAG TTTGTTACAA AAAAGCGCTA TTGGAAGTAG GG 2325 // GAAAGGAGGA CAAAATACAG TTAAAGAGGG TAAGGAGAGA TTCCAGGTCT AATTTTACTT ACCGGGCACA AGCCATTGGC GGTTCCACTT GCTTGAGCTT TTTAAGGTAT 2280 AAGTGTTGAA TTGGAGTGAG TGCAGTCATA 1920 2160 1980 2100 2040 1860 1800

// ATG GTG AAG GTA AAG AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAG ATA AAG AAG GTT GAC ATG TTA GCA AGA GCA GAA AGA GAG AAA TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT TTC AAG TAT AAG GGT GAA GAG AAA // TGA GAA GTA GAC AAA GAC ACT GAA TTA GAC TCA

Deep Vent- Sac7d DNA polymerase fusion protein (Fig. 17-AAA)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

AAAGAAAACG GCGAGTTTAA GGTTGAGTAC ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG GTCGAGGTAG AGCTATGCTG GACATAGAAA CCCTCTATCA CGAAGGGGAG CTAATAGACA AAGGCCTAAT TCCAATGGAA GGCGATGAAG AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGGTATGGA GGCTGTACTT TGAACACCCT AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGG TACCACGTGA TTAGGAGAAC GATAAACCTC ATGCAGAGGC AAGAGGCCG AAAGATCCCG TTGGGGATAT GACAGCGGTG AAAAGCTCGG GATAAAGCTA CCCCTGGGAA GGGACGGTAG ATGTTATAAT TACCTACAAC TTTCCAGCGA GAGGGAGATG ATAAAGCGGT ATGAGGAAGA AGCCAAAGTC CCAACATACA CCCTCGAGGC AGTTTATGAG GAGATAAAGG GGCGATTCTT ATAACGTGGA GAGTTCGCGA GAGTACGACA CAGGACXXXC CCGCAATAAG GACAGAAACT TCGACCTTCC TCCTCAAGGT GATAAGGGAG AAAAGATCGA AGGGGCCCAT TATAATGATA AGCTCAAGTT TTCCGTTCGC AGTTCCTGGG GAGGCCGATT TTAGACCTTA CATTTACGCT GAAGGATACA TGAGCCAAAG CTATCTAGTI TCTCCCGTAC GCTCGCATTT GAAGAGGTAC GGATAAGATA CTTTGACCTC 660 600 540 480 420 360 300 240 780 720 180

Fig. 17 AAA (cont)

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GCCGTTCTTA
                            TTCGATCTCA
                                           GGGTACATAG
                                                        GITGCCGTGG
                                                                                    GTAAAGATAG
                                                                                                 AAAGAAACCC
                                                                                                                GAAGGGAAGA
                                                                                                                              GAGGGCTTCT
                                                                                                                                             GCCCTAGAGT
                                                                                                                                                          GACACAGATG
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                                                                                                                                                                                    GCAAAAGCCC
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 ACTAAACAGA
                                                                       CTAGTTATTT
                                                                                                                                                                                                                               AAGTTCTGCA
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CAGGTCTTAC GGCATGGCTT AACATCAAGA AGAAG //
             GAATATTAGA GGCCTTTGGG TACAGGAAAG AAGACCTCAG
                           GGAAGCATAA GTATGACGCT
                                         TGCTGAGGGG AGACGGGCCA
                                                                                                                                                                      TCGTAAGGAA GGAACTGGAG
                                                                                                                                                                                                                TAAAAAGGAA
                                                        CAAAAAGGTT
                                                                     ACGAGCAGAT CACGAGGCCC
                                                                                   TTAAGGAGGT
                                                                                                 AAGCAAAAGT CCTAGAGGCT
                                                                                                               TAATCACTAG
                                                                                                                              ACGTGAGAGG
                                                                                                                                                         GACTCTACGC CACAATTCCT
                                                                                                                                                                                    GTTGGTACTG TAAGGAGTGC
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                                                                                                                                                                                                                              AGGACTTCCC
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                            GAGTATTACA
                                        ATAAGCAAGA GGGCTATCCT TGCAGAGGAG
                                                      GGAGTAAAGG TGAGGCCTGG
                                                                    CTTCACGAGT ACAAGGCTAT
                                                                                                 ATCCTAAAGC
                                                                                                                                                                      GAAAAGTTCG
                                                                                  CTGAGCAAGT
                                                                                                               ATAGTCAGGA
                                                                                                                                                        GGGGCAAAAC
                                                                                                                                                                                    GCAGAGAGCG
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                                                                                                                              ACGAAGAAGA
                                                                                                                                           AAGCTCCCAG
                                                                                                                                                                                                                TCTAAAGACC
                                                                                                                                                                                                                              CCCAGCCTGC
                                                                                                                                                                                                                                            GAATACGATG
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                                                                                                                                                                                                                                                          CCCTCGATAA
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                           TAGAAAATCA
                                                                                  ACGAAATACC
                                                                                                                                          GGCTGTTGGA GCTTGAGTAC
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                                                                                                ATGGCAACGT
                                                                                                               GGGACTGGAG
                                                                                                                            AGTATGCGTT
                                                                                                                                                       CCGAGGAGAT
                                                                                                                                                                      GGTTCAAAGT
                                                                                                                                                                                   TTACGGCCTG
                                                                                                                                                                                                                CAATCGAGAA
                                                                                                                                                                                                                             TCAAGAGGTT
                                                                                                                                                                                                                                            TCGCCCCAGA
                                                                                                                                                                                                                                                         TAATCACCCA
                                                                                                                                                                                                                                                                                    AGAGGGAGTA
              GTGGCAGAAC
                          GGTTTTACCI
                                                      CATGGTGAT
                                                                                                                                                       AAAGAAGAAA
                                                                    AGGTCCGCAC
                                                                                  TCCAGAAAAG
                                                                                                               CGAAATAGCC
                                                                                                                             GATAGATGAG
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                                                                                                                                                                                   GGGGAGGGAA
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                                                                                                                                                                                                                GAAGATGCTI
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                                                                                                                                                                                                                                                                                                  CCTCAGGAAG
                                                                                                                                                                                                                                                                                                                CGGCCAGCCC
                                                                                                                                                                                                                                                                                                                               GGTAACGTAC
                                                                                                TGAGGAGGCA
                                                                                                                                                                                                                                            GGTTGGGCAC
                                                                                                                                                                                                                                                          TAACGTCTCA
                                                                                                                                                                                                                                                                                     CGAGAGAAGG
                                        2160
                                                      2100
                                                                    2040
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             2280
                           2220
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                                                                                                                                                                                                                                                                                                  1080
2328
                                                                                                                                                                                                                                                                                                                1020
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// ATG GGT GIG ATA AAG AAG AAG ATG TTA ACA GGT AGA GGA GCT GTA AAG GTA GIT GCA AAG AGA TGG AGA GTA TTC AAG TAT AAG GCA GAA . GGC AGA AGC GAG AAA GGT GAA GAG AAA ATG GAG AAA GAT GCT AAG AAA GIG TCC TTT GAA ACC GTA CCA AAA GAC TAT GAA TTA GAC GAC ACT TCA

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V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)
                                                                       V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)
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AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA $^\prime /$ ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TCA TTA GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG AAA // AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC

GTCGAGGTAG TTTCCAGCGA GAGGGAGATG ATAAAGCGGT TCCTCAAGGT GATAAGGGAG GCCTACGAGA GCAATCTTCG ATGCAGAGGC **AAGAGGGCCG** AAAGATCCCG AGCTATGCTG GACATAGAAA CCCTCTATCA CGAAGGGGAG GAGTTCGCGA AGGGGCCCCAT TATAATGATA CTAATAGACA AAGGCCTAAT TCCAATGGAA GGCGATGAAG AGCTCAAGTT GCTCGCATTT AGAGAGCATT CCGCAGTTAT TGACATCTTT GAGTACGACA TTCCGTTCGC GAAGAGGTAC GAGGTATGGA GGCTGTACTT TGAACACCCT CAGGACXXXC CCGCAATAAG GGATAAGATA AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA AGTTCCTGGG GAGGCCGATT CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGG AAAGAAAACG GCGAGTTTAA'GGTTGAGTAC GACAGAAACT TTAGACCTTA CATTTACGCT CCGGATACGC CTAAGGGAGA CTGTGGGATG GAGCTCGGTA ACTGGAAAGG TACCACGTGA GATTACAGGC TTAGTTTCCC //ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG AGGACTTCCC GCTACGCTGG GGGATACGTT GGAATGAATT GGCTCCAAAC AAGCCGGATG AGAGGGAGTA TTTCTAGGTC TTCAACTGGC AACTTGGTGG AGTGGTACCT CCTCAGGAAG GGGAGTTCTT CCCAATGGAG GCCCAGCTTT CAAGGTTAGT CGGCCAGCCC GACTGGAGAG AGTTGCAAAG TATTCAATGG GAAAGCCAAA GGAGAAAGTT AAAAGCTCGG GATAAAGCTA CCCCTGGGAA GGGACGGTAG TGAGCCAAAG ATGTTATAAT TACCTACAAC GGCGATTCTT TCGACCTTCC ATGAGGAAGA AGCCAAAGTC ATAACGTGGA AAAAGATCGA TCTCCCGTAC AACGGGCAAT CAAAATCCTG TAAAAAGGAA GATGAAAGCT TGAACAGGGA AGGGTGTAGG TAGATTTCAG GAGCCTGTAC TTAGGAGAAC GATAAACCTC CCAACATACA CCCTCGAGGC AGTTTATGAG TTGGGGATAT GACAGCGGTG GAGATAAAGG GAAGGATACA GGGGTTTATC CCCTCGATAA TAATCACCCA AAGGAGCCGG AGAAAGGGCT GCAAACAGCT GAATACGATG TACGCTCACG AGATAGCTGA GGCCTGGGAG CCCAGCCTGC TCTAAAGACC CAATCGAGAA TCAAGAGGTT ATTGGATGAA TCGCCCCAGA AGGATGCAAA GGTAACGTAC ATTATGGGTA TAACGTCTCA CTGGGAGGGG GAAGATGCTI GGTTGGGCAC CGAGAGAAGG CTTTGACCTC CTATCTAGTT 1380 1320 1260 1200 1140 1080 1020 960 900 840 780 720 660 600 540 480 240 360 300 180

				2.0	£	
2328		AGAAG TAA	AACATCAAGA	ACTAAACAGA CAGGTCTTAC;GGCATGGCTT AACATCAAGA AGAAG TAA	CAGGTCTTAC	ACTAAACAGA
2280	GTGGCAGAAG	AAGACCTCAG	TACAGGAAAG	GCCGTTCTTA GAATATTAGA GGCCTTTGGG TACAGGAAAG AAGACCTCAG GTGGCAGAAG	GAATATTAGA	GCCGTTCTTA
2220	GGTTTTACCT	TAGAAAATCA	GAGTATTACA	TTCGATCTCA GGAAGCATAA GTATGACGCT GAGTATTACA TAGAAAATCA GGTTTTACCT	GGAAGCATAA	TTCGATCTCA
2160	TGCAGAGGAG	GGCTATCCT	ATAAGCAAGA	GGGTACATAG TGCTGAGGGG AGACGGGCCA ATAAGCAAGA GGGCTATCCT TGCAGAGGAG	TGCTGAGGGG	GGGTACATAG
2100	CATGGTGATA	TGAGGCCTGG	GGAGTAAAGG	GTTGCCGTGG CAAAAAGGTT AGCCGCTAGA GGAGTAAAGG TGAGGCCTGG CATGGTGATA	CAAAAAGGTT	GTTGCCGTGG
2040	AGGTCCGCAC	ACAAGGCTAT	CTTCACGAGT	CTAGTTATTT ACGAGCAGAT CACGAGGCCC CTTCACGAGT ACAAGGCTAT AGGTCCGCAC	ACGAGCAGAT	CTAGTTATTT
1980	TCCAGAAAAG	ACGAAATACC	CTGAGCAAGT	GTAAAGATAG TTAAGGAGGT AACTGAAAAG CTGAGCAAGT ACGAAATACC TCCAGAAAAG	TTAAGGAGGT	GTAAAGATAG
1920	TGAGGAGGCA	ATGGCAACGT	ATCCTAAAGC	AAAGAAACCC AAGCAAAAGT CCTAGAGGCT ATCCTAAAGC ATGGCAACGT TGAGGAGGCA	AAGCAAAAGT	AAAGAAACCC
1860	CGAAATAGCC	GGGACTGGAG	ATAGTCAGGA	GAAGGGAAGA TAATCACTAG GGGGCTTGAA ATAGTCAGGA GGGACTGGAG CGAAATAGCC	TAATCACTAG	GAAGGGAAGA
1800	GATAGATGAG	AGTATGCGTT	ACGAAGAAGA	GAGGGCTTCT ACGTGAGAGG GTTCTTCGTG ACGAAGAAGA AGTATGCGTT GATAGATGAG	ACGTGAGAGG	GAGGGCTTCT
1740	GCTTGAGTAC	GGCTGTTGGA	AAGCTCCCAG	GCCCTAGAGT TCGTAGATTA TATAAACGCC AAGCTCCCAG GGCTGTTGGA GCTTGAGTAC	TCGTAGATTA	GCCCTAGAGT
1680	AAAGAAGAAA	CCGAGGAGAT	GGGGCAAAAC	GACACAGATG GACTCTACGC CACAATTCCT GGGGCAAAAC CCGAGGAGAT AAAGAAGAAA	GACTCTACGC	GACACAGATG
1620	CTTATACATA	GGTTCAAAGT	GAAAAGTTCG	TATATAGAGT TCGTAAGGAA GGAACTGGAG GAAAAGTTCG GGTTCAAAGT CTTATACATA	TCGTAAGGAA	TATATAGAGT
1560		TTACGGCCTG	GCAGAGAGCG	GCAAAAGCCC GTTGGTACTG TAAGGAGTGC GCAGAGAGCG TTACGGCCTG GGGGAGGGAA	GTTGGTACTG	GCAAAAGCCC

JDF-3 - Sac7d fusion protein (Fig. 17-CCC)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

 $A \verb|TGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGGAGTACATCGAAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAAAGTCCT$ $\tt GCGCTTCTTGAGGGTCGTTAAGGAGGACCCCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAGCGCTGTGAGAAGCTTTGCCGTGAGCTTT$ ${ t ACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGGTGAAGAAAAGTTCCTCGG$ $\tt GTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAgcGTTTGGCCGCCAGAGGT$ ACTGGAGCGAGATAGCGAAGGAGACGCAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAA $\tt CTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTC$ $\mathsf{CAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCCCATCAAGATTCTCGCCAACAGCTACTACGGCTACTACGGCT$ $\tt GGTCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCC$ $\mathtt{ACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACACGGCGAGGGGCTTGAGAG$ $\mathtt{ACCCTCGGGAGGGAGCGAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAA$ ${ t TTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTC{GAC}$ A ${ t TCGAGACCCTCTACCACGAGGGAGAAGAGTTTGGAA}$

j

 $\textbf{GTTAAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATG$ GTGGCTGAAGCCGAAGGGAAGAAGAAG//

// ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT ATA AAG GAC ATG AAG AAG GTT TGG AGA GTA GGC TTA GCA AGA GCA GAA AGA GAG AAA // TAG ACA GGT AGA GGA GCT GTA AGC AAA ATG GAG AAA GAT GCT CCA AAA GAA TTA GTG TCC TTT ACC TAT GAC TCA GAC

Sac7d - JDF-3 fusion protein (Fig. 17-DDD)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)
V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

// ATG GTG AAG GTA AAG TTC AAG TAT AAG GGT GAA GAG AAA GAA GTA GAC ACT TCA AAT GGT AAG ACA GGT AGA GGA GCT GTA AGC GAG AAA GAT GCT CCA AAA GAA TTA AAG ATA AAG AAG GTT TGG AGA GTA GGC AAA ATG GTG TCC TTT ACC TAT GAC GAC GAC ATG TTA GCA AGA GCA GAA AGA GAG AAG AAA //

 $\mathtt{AGGGTCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTT$ AAACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCGGCGAGGGGGCTTGAG ${\tt CCTTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTCGACGACGACGCTCTACCACGAGGGAGAAGAGTTTGC$ GGCAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACCCGCAGGACXXXCCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGGGGTCATCGACATCTACGAGTACGACATAC CTACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGGTGAAGAAAAGTTCCTC $\mathtt{AAGTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAgcGTTTGGCCGCCAGAG$ CGACTGGAGCGAGATAGCGAAGGACGCAGGCGAGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCGTCAGAAATTGTCAGGGAAGTCACCGAAAAAGCTGAGC ${ t TCGAACTCGAATACGAGGGCTTCTACGTCAGGGGCCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGGCAAGATAACCACGCGCGGGCTTGAGATAGTCAGGCG$ $\tt CTCTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTC$ $\tt CTATGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAAAGTC$ ${\tt GGCAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGGCTGGAGAAGAATCTCCTCGATTACAGGCAACGC{\tt GCC}$ ${\tt TTACCCTCGGGAGGGAGCGAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCAT$ $\mathtt{AAGCGCTTCTTGAGGGTCGTTAAGGAGGAGCACCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAGCGCTGTGAGAAGCTTGGCGTGAGCT$ //ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCGTCATCAGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAGCCCTACTT

Fig 17 DDD (cont)

GCGTGGCTGAAGCCGAAGGGGAAGAAGAAGTGA ${\tt TGCGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGC$ $\tt GTGTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGA$

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Synthetic Sso7d gene: (Fig. 17-EEE)

A T V K F K Y K G E E K E V D I S K GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA AAA GAG GTA GAC ATC TCC AAG

I K K V W R V G K M I S F T Y ATC AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GAG Ħ _ GGC

G G K T G R G A V S GGT GGC AAG ACC GGC CGT GGT GCG GTA AGC E K D A P K E L GAA AAG GAC GCG CCG AAG GAG CTG

L Q M L E K Q K K
CTG CAG ATG CTG GAG AAG CAG AAA AAG

Sso7d-Taq DNA polymerase fusion protein (Fig. 17-FFF)

// A T V K F K Y K G E E K E V D I S K // GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC AAG

I K K V W R V G K M I S F T Y D E G ATC AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GAG GGC

G G K T G \hat{R} G A V S E K D A P K E L GGT GGC AAG ACC GGC CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG CTG

L Q M L B K Q K K // G G G CTG CAG ATG CTG GAG AAG CAG AAA AAG // GGC GGC GGT

GIC T S C ACT AGT M L P L F E P K G R V L L ATG CTG CCC CTC TTT GAG CCC AAG GGC CGG GTC CTC CTG GIG V

D GAC H L A Y R T F H A L K G L T CAC CTG GCC TAC CGC ACC TTC CAC GCC CTG AAG GGC CTC ACC ACC

S AGC × ٢ >ഥ U V Q A X Y G F A K GTG CAG GCG OTC TAC GGC TTC GCC AAG Q U Þ < Н < 긔 D S AGC

> K AAG

FIG. 17 FFF (CONT)

ရှိ ရ CIG CIG a GCC GCC acc A CIC D GAC A GCC CIG E GAG DOI S K K PAG AAG I D GAC CTT A GCG GAC GAA DDD 4 H CAC CIC I ATC TTC F CICGGG G CIC CGC S TAC T ACC Y TTT CGC R AAG ეეე ტ E GAG CAG 222 4 D GAC K AAG R CGG 500 P A E K E G GCG GAA AAG GAG GGC CIC H CAC GAG CTC CTT GAT N AAC E A GAG GCC AAG K AAG A GCC A GCC E V P G Y E A D D V GAG GTC CCG GGC TAC GAG GCG GAC GAC GTC R Q L A L I K E L V CGG CAA CTC GCC CTC ATC AAG GAG CTG GTG GAC L K CTG AAG L D CTG GAC A GCC T A R K ACG GCG AGG AAG CIG D GAC TGG GGG CIG Y TAC TTC T ACC CIT TCC S CTT GAC GCG GTG ATC CIC CGG R GAC E GAG A GCC GGG G 9 9 9 E GAG W TGG Y E V TAC GAG GTC L K CTG AAG E GAG TCC S D GAC R I H CGC ATC CAC 9 9 9 AAA AAA E GAA CIT TGG A GCC R AGG E GAG K AAG Y TAC CIG D GAC D P S DDD P S AGC CGG R TAC K AAG GTG GTC TTT GAC GCC AAG GCC acc A D N GAC AAC R I A G GCG GGC E GAG CIG E GAG မရင် ရေင GTC V TGG CIC I R E GAG DDD d A GCC D P CIG CIC 999 4 TGG D GAC AAG CIT CIC CGG H CAC AGG 999 P D L GAC CTC A P GIG ୍ଦ୍ର ଜୁନ L A E GAG ეეეე ტ CGG R 222 4 ၁၁၁ a T ACC GAA GCC CGC # S AGC E GAG K AAG 999 9 E GAG D GAC . GGG I ATC CIG GTC V ය යෙ D GAC CIG AGG R CAG T ACG A GCC CIT D GAC CIG gaa AAG X Y TGG AAA AAA CIG 6 6 6

A GCC CGC R 9 9 TAC GCC A TCC E GAG A GCC CIC N L AAC CTG L L D P S N T T P E G V A R R Y G CTC CTG GAC CCT TCC AAC ACC ACC CCC GAG GGG GTG GCC CGG CGC TAC GGC CIG V G F V L S R K E P M W A GTG GGC TIT GTG CTT TCC CGC AAG GAG CCC ATG TGG GCC cgg R CGC R QTG R AGG E W T E E A G E R A A L S E R L GAG TGG ACG GAG GCG GCG GAG CGC GCC CTT TCC GAG AGG CTC E GAG GCC A D GAC CIC R AGG E GAA R AGG acc A Q CAG E GAG GTG W G R L E G E E R L L W TGG GGG AGG CTT GAG GGG GAG AGG CTC CTT TGG P L A E V GCC GAG GTC A Y GCC TAT 9 9 R AGG T Y I D
ACC TAC ATT GAC AAG ACC GGC AAG CGC TCC ACC CIG K AAG CTT G G R V H R
GGG GGC CGG GTC CAC CGG E R V GAA AGG GTC E GAG I V E ATC GTG L R 9 9 S A V A GCG L P TTC CGG R ეეეე ტ GCC e Gag CIC မရ ရ R CGC K I HAG ATC TTG TIG TTT CIG CIG CTT P G D D CCC GGC GAC GAC වටට අ D GAC A GCC TCC A H M E GCC CAC ATG GAG CIC S A A S AGC GCC GCC D L GAC CTC GCC A E L G GAG CTA GGG 990 9 CIG A P E HCAC E GAG cag K Aaa I ATC 222 P ote V ეეე გ D GAC GTC CTT A GCC A GCC M ATG CCI CAC CCC TTC L Y Y K A ეეე გ E GAG T ACG S V AGC GTT N AAC CIC A GCC မှ မရှိ CIC CIC I GCC A I V GTG CGG TTC A GCC N AAC

OTC S AGC org V CCI P TTC GTC CAG CGC R S 9 9 9 T ACC CGG R TAC ეეე ტ I ATA cee R TTC A GCC E GAG QTC E GAG CIC A GCC CGG R CAG N L : AAC CTC E GAG W TGG TIC CIC E GAG GAG. CIC F TTC CIC GAG I ATT A GCC Y A GCC I ATC ATG A GCG 999 999 R AGG Q CAG 9 9 9 E GAG A GCC Q CAG CIC AAG A GCC CGC R V GTG org V caa R A GCC MTG K AAG AAC CIT CIG CGC R D GAC E GAG AAC E GAG D GAC CIG AGCT T ACC TTC TCG ATC Q CAG CGC R D D E GAG CGC R A GCC I I ATT GCC A CIG M ATG Y 999 9 CIG CAC H CAC 222 P A T GCC ACG E GAG CAC E GAG OI.C GCC A GIG M ATG T ACG CIC TGG OTC R T AAG X E GAG CGC CGC R CGC R TTC P CCA E GAG S CTA GAC 999 9 Y TAC CIC CGG E GAG CIC T ACC N AAC ეგე ი TIG CTG TTC M ATG R AGG TTT A GCG CTA DOL A GCC GIG P P D GAC A GCC DDD 4 222 d E GAG R AGG CAG Q CAG S E GAG acc A CII AGG AGG GTC V A GCC CGG R S AGC E GAG K AAG TGG W CIG T AAC AAC CTA . G G CAG TTC ACC T D GAC ATG CIG E GAG GGC G OTC A Y TAC 555 A A GCC I TTC I ATC Y

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CCA AAA GAG AGG GCG GAG GCC GTG GCC CGG CTG GCC AAG GAG GTC ATG GAG GGG

Y P L A V P L E V E V G I TAT CCC CTG GCC GTG CCC CTG GAG GTG GAG GTG GGG ATA S A K E G I D G R G G G H H H H H TCC GCC AAG GAG GGC ATT GAT GGC CGC GGC GGA GGC GGG CAT CAT CAT . GGG D W

Pfu DNA Polymerase (WT) -Sso7d fusion protein (Fig. 17-GGG)

CAT CAT TAA

ccctggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat caactcttaca cctcccctat tttctcttt atgagatttt taagtatagt tatagagaag gttttatact ccaaactgag ttagtagata tgtgggagc ataatgattt tagatgtgga gttttatact ccaaactgag ttagtagata tgtggggagc ataatgattt tagatgtgga ttacataact gaagaaggaa aacctgttat taggctattc aaaaaagaga acggaaaatt taaggtataga gctcttctca gggatgattc aaaagattgaa gaagttgaga aaaataacggg ggaaaggcat ggaaaggttg tgagaacttta ttggagacat ccccaagatg ttcccactat tagagaaaaa gttagagaac atccagcagt tgtgggacat ttcgaatacg atattccatt tgcaaaggag ttccaccatt ttcgaaagga tacctcatcg acaaaggcct aataccgagg gaagagggaag aagagctaaa gattcttgcc ttcgatatag aaaccctcta aataccgaagga gaagaggttg gaaaaggccc aattataatg attagttatg cagatgaaaa

Fig. 17 GGG (cont)

gtcaagcaca gggaaccttg tagagtggtt cttacttagg aaagcctacg aaagaaacga aggtggattc gttaaagagc cagaaaaggg gttgtgggaa aacatagtat acctagattt agtagctcca aacaagccaa gtgaagagga gtatcaaaga aggctcaggg agagctacac ccttccaatg gaaattcagc tttcaagatt agttggacaa cctttatggg atgtttcaag gagagttgcc aaatactcga tggaagatgc aaaggcaact tatgaactcg ggaaagaatt aaaggagaag gtatacgccg acgagatagc aaaagcctgg gaaagtggag agaaccttga gacaataaat ctcccaacat acacactaga ggctgtatat gaagcaattt ttggaaagcc tatgacggct gtagaagtca agggaagaat acatttcgac ttgtatcatg taataacaag agttacttat aatggagact cattcgactt cccatattta gcgaaaaggg cagaaaaact cgagagagag atgataaaga gatttctcag gattatcagg gagaaggatc ctgacattat tgaagcaaag gtgattactt ggaaaaacat agatcttcca tacgttgagg ttgtatcaag gaaggagctc gaagaaaagt ttggatttaa agtcctctac attgacactg atggtctcta gataaaactc ttagcaaatt ctttctacgg atattatggc tatgcaaaag caagatggta aaaaatgaag gaaactcaag atcctataga aaaaatactc cttgactata gacaaaaagc ccctggtttt ataccaagtc tcttgggaca tttgttagag gaaagacaaa agattaagac tgagggatgc aagaactatg atatcgctcc tcaagtaggc cacaagttct gcaaggacat tagageceta tatecetega ttataattae eeacaatgtt tetecegata etetaaatet tgggattaaa ttaaccattg gaagagatgg aagcgagccc aagatgcaga gaataggcga atacataaat tcaaagctcc ctggactgct agagcttgaa tatgaagggt tttataagag tgcaactatc ccaggaggag aaagtgagga aataaagaaa aaggctctag aatttgtaaa ctgtaaggag tgtgctgaga gcgttactgc ctggggaaga aagtacatcg agttagtatg

tig 17 GGG (cont)

cagagaattg agaaacatca gaaactttga cttctacaac atttctaact ttgcaactct ctatctgcat gcttctctgg ctcggaanng gaggattcat aacaacagta tcaacattct caagtatgac gcagaatatt acattgagaa ccaggttctt ccagcggtac ttaggatatt aggcgatggt ccaattagca_atagggcaat tctagctgag gaatacgatc ccaaaaagca gataacaaga ccattacatg agtataaggc gataggtcct cacgtagctg ttgcaaagaa agtaatacaa aagcttgcca attatgaaat tccaccagag aagctcgcaa tatatgagca agttttggag acaatactaa aacacggaga tgttgaagaa gctgtgagaa tagtaaaaga gggattcttc gttacgaaga agaggtatgc agtaatagat gaagaaggaa aagtcattac aaagtataat ttcaagctcc ttcttcttca tgacagatga aaaattgttt tgtctctttt acaatttttt ccttgtatct cctaatgtat aagcaagcca aaggagagta gatgctacct tcaagatttt ctaaaagaat tttaacggcc tcctcgtcaa tttcgacgac gtagatcttt tttgctaagt gaatagaata aacaacatca ctcacttcaa acgccttcgt tagaaatggt tgggtaatta aaaacccatg ctcttgggag aatcttcgaa taaaatccct aacttcaggc tctaaccttt ttctatgaaa gaagaactga gcaggaatta ccagttcttc cgttatttta aacttcctgg cttaacatta aaaaatccta gaaaagcgat agatatcaac ttttattctt actagctgct aaaggagtta aaataaagcc aggaatggta attggataca tagtacttag tcgtggttta gagatagtta ggagagattg gagtgaaatt gcaaaagaaa ctcaagctag ttccgggagt tttgtattgc tctagccaag gtttgggatt tttgaatcct ttaactctgg tttgctccaa gcagagccgc tccaatggat aacacccctg ttcccgcacc caagtccgct

aagattgaga tgttcttgg // gtagaagcgg cactactcag atgcttcccc aggaatgagg ttgttgtagc tcntcccnga gacactcaaa taccagacga caatggtgtg ctcactcaag ccccatatgg gttgagaaaa ctcgatttcc ttggttgtgc tccatatgat aagcttccaa agtgggtgtt cagactttta taacttttac agaaataact gtctcaaatt atgacaactc ttgacatttt tacttcatta ccagggtaat gtttttaagt atgaaatttt tctttcatag aggaggnnnn nngtcctctc // GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC AAG ATC AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GAG GGC CTG CAG ATG CTG GAG AAG CAG AAA AAG GGT GGC AAG ACC GGC CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG CTG // TGA

PFU DNA POLYMERASE (V93 R OR E)-Sso7d fusion protein (Fig. 17-HHH)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

ATGCAGAGAA GGAGACTCAT GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GAGTTTGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC GATAGAACTT TTAGACCATA CATTTACGCT ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TAATTACCCA 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACAGG ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA CTCATCGACA AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC TCGCATTCCC ATATTTAGCG 660 AAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGGA AAGGCATGGA CGAGCCCAAG AGCCTGGGAA TCTTCCATAC 540 360 180 720

GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC 2328 ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA TGGTTTAGAG ATAGTTAGGA GAGATTGGAG

// GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC GGT GGC AAG ACC GGE CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG ATC AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC CTG CAG ATG CTG GAG AAG CAG AAA AAG // TGA TCC GAG CIG GGC AAG

PFU DNA POLYMERASE (G387P/V93R OR E) -Sso7d fusion protein (Fig. 17-III)

G387P Mutant (CCN is the codon for Proline where N = C, G, A, or T)

AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA ACTCAAGATC ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA AGTGGAGAGA ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GTTGAGGTTG TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT GAGTTTGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAGAT TCTTGCCTTC 420 GATATAGAAA CCCTCTATCA CGAAGGAGAA CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT GGAACATCCC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA GGAGCTCGAA CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT 1500 CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 AAGTTCTGCA ${f N}$ GGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATTA TA ${f A}$ TTACCC ${f P}$ 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA GCTACACACC 180 720 540 360

GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTATGACGCA ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT 2100 ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA GAGATTGGAG GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT AAAGAAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG ATACCAAAAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA TTACATGAGT TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 GTGAGAATAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT ATAAGAGGGG 2328

// GCA ACC ATC AAG GGT GGC AAG AAA GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC CIG ACC GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GGC CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG GAG AAG CAG AAA AAG // TGA // TGA GAG GGC TCC AAG CIG

PFU DNA POLYMERASE (D141A/E143A/V93R OR E) -Sso7d fusion protein (Fig. 17-

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE

D141A/E143A Mutant (GCN is the codon for alanine where N = C, G, A, or T)

CTCATCGACA AAGGCCTAAT ACCAATGGAG GGGGAAGAAG AGCTAAAAGAT TCTTGCCTTC 420 GCNATAGCNA CCCTCTATCA CGAAGGAGAA GAGTTTGGAA AAGGCCCAAT TATAATGATT 480 AGTTATGCAG ATGAAAATGA AGCAAAAGGTG ATTACTTGGA AAAACATAGA TCTTCCATAC CAAGATXXXC CCACTATTAG AGAAAAAGTT 300 AGAGAACATC CAGCAGTTGT GGACATCTTC GAATACGATA TTCCATTTGC AAAGAGATAC GATAGAACTT TTAGACCATA CATTTACGCT 120 CTTCTCAGGG ATGATTCAAA GATTGAAGAA GTTAAGAAAA TAACGGGGGA AAGGCATGGA ATGATTTTAG ATGTGGATTA CATAACTGAA GAAGGAAAAC CTGTTATTAG GCTATTCAAA 60 AAAGAGAACG GAAAATTTAA GATAGAGCAT V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) AGTGGAGAGA CCAACATACA CACTAGAGGC TGTATATGAA 840 GCAATTTTTG GAAAGCCAAA GGAGAAGGTA TACGCCGACG AGATAGCAAA AGCCTGGGAA 900 ATGCAGAGAA TAGGCGATAT GACGGCTGTA GAAGTCAAGG GAAGAATACA TTTCGACTTG 780 TATCATGTAA TAACAAGGAC AATAAATCTC GGAGACTCAT TCGCATTCCC ATATTTAGCG 660 AAAAAGGGCAG AAAAACTTGG GATTAAATTA ACCATTGGAA GAGATGGAAG CGAGCCCAAG GTTGAGGTTG AAGATTGTGA GAATTGTTGA TGTAGAGAAG GTTGAGAAAA AGTTTCTCGG CAAGCCTATT 240 ACCGTGTGGA AACTTTATTT AAGTTCTGCA AGGACATCCC TGGTTTTATA CCAAGTCTCT TGGGACATTT GTTAGAGGAA 1380 AGACAAAAGA TTAAGACAAA AATGAAGGAA TAATTACCCA CAATGTTTCT 1260 CCCGATACTC TAAATCTTGA GGGATGCAAG AACTATGATA TCGCTCCTCA AGTAGGCCAC 1320 GCTACACAGG.TGGATTCGTT AAAGAGCCAG AAAAGGGGTT GTGGGAAAAC 1200 ATAGTATACC TAGATTTTAG AGCCCTATAT CCCTCGATT! 1080 GCCTACGAAA GAAACGAAGT AGCTCCAAAC AAGCCAAGTG AAGAGGAGTA TCAAAGAAGG 1140 CTCAGGGAGA ATTCAGCTTT CAAGATTAGT TGGACAACCT 1020 TTATGGGATG TTTCAAGGTC AAGCACAGGG AACCTTGTAG AGTGGTTCTT ACTTAGGAAA TATCAAGCGA GAGAGAGATG ATAAAGAGAT TTCTCAGGAT TATCAGGGAG 600 AAGGATCCTG ACATTATAGT TACTTATAAT ACCTTGAGAG AGTTGCCAAA TACTCGATGG AAGATGCAAA GGCAACTTAT 960 GAACTCGGGA AAGAATTCCT TCCAATGGAA GGAACATCCC 540 720 360

ACTCAAGATC CTATAGAAAA AATACTCCTT 1440 GACTATAGAC AAAAAGCGAT AAAACTCTTA GCAAATTCTT TCTACGGATA TTATGGCTAT

1500 GCAAAAGCAA GATGGTACTG TAAGGAGTGT GCTGAGAGCG TTACTGCCTG GGGAAGAAAG 1560 TACATCGAGT TAGTATGGAA

GTATGACGCA GAATATTACA TGGAGAACCA GGTTCTTCCA 2220 GCGGTACTTA GGATATTGGA GGGATTTGGA TACAGAAAGG AAGACCTCAG 2100 GGATACATAG TACTTAGAGG CGATGGTCCA ATTAGCAATA GGGCAATTCT AGCTGAGGAA 2160 TACGATCCCA AAAAGCACAA GTGAGAATAG TAAAAGAAGT AATACAAAAG CTTGCCAATT ATGAAATTCC ACCAGAGAAG 1980 CTCGCAATAT ATGAGCAGAT AACAAGACCA CAGATTGGAG TGAAATTGCA 1860 AAAGAAACTC AAGCTAGAGT TTTGGAGACA ATACTAAAAC ACGGAGATGT TGAAGAAGCT 1920 ATAAGAGGGG ATTCTTCGTT ACGAAGAAGA GGTATGCAGT AATAGATGAA 1800 GAAGGAAAAG TCATTACTCG TGGTTTAGAG ATAGTTAGGA AAAGAAAAAG 1680 GCTCTAGAAT TTGTAAAATA CATAAATTCA AAGCTCCCTG GACTGCTAGA GCTTGAATAT 1740 GAAGGGTTTT GGAGCTCGAA GAAAAGTTTG GATTTAAAGT CCTCTACATT 1620 GACACTGATG GTCTCTATGC AACTATCCCA GGAGGAGAAA GTGAGGAAAT ATACCAAAAG 2280 ACAAGACAAG TCGGCCTAAC TTCCTGGCTT AACATTAAAA AATCC // TTACATGAGT ATAAGGCGAT AGGTCCTCAC 2040 GTAGCTGTTG CAAAGAAACT AGCTGCTAAA GGAGTTAAAA TAAAGCCAGG AATGGTAATT

// GCA ACC GTA AAG :TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC GGT GGC AAG ATG AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GAG CTG GAG AAG CAG AAA AAG ACC GGC CGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG CIG GGC AAG

KOD DNA POLYMERASE - Sso7d fusion protein (Fig. 17-KKK)

GACATTGAAA CTCTCTACCA GAGGTCTGGA AACTCTACTT TACTCATCCG CAGGACXXXC CAGCGATAAG CGAGAGCATC CAGCAGTTAT TGACATCTAC GAGTACGACA TACCCTTCGC ACGGTTGTAA CGGTTAAGCG CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA AAGGAAAACG GCGAGTTTAA ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) GTTGACGTCG AGCTACGCCG CTCATAGACA AGGGATTAGT GCCAATGGAA GGCGACGAGG GCCGTCTTCG GTCAGCCGAA TATCCTGTGA ATTCAGAGGA AAGCGCTGTG AAAGACCCGG TGGGCGACAG TAAGACGGAC AAAAGCTCGG ACGTTCTCAT TCTCGACGGA GAGGGAGATG ATAAAGCGCT TCCTCCGTGT TGTGAAGGAG 600 ACGAGGAAGG GATAAACCTG CCCACATACA GGAGAAGGTT TACGCTGAGG AAATAACCAC AGCCTGGGAA GTTTGCCGTC GAAGTGAAGG AATAAACTTC GCCCTCGGAA AACCTACAAC GGCCAGGGTG ATAACTTGGA TGAGGGCGAG GAGTTCGCCG GGTTGAAAAG GTTCAGAAGA GATTGAGTAC GACCGGACTT GGCGACAACT TACTCGATGG CGCTTGAGGC GACGGATACA GGGATGGAAG TCGACTTCGC AGAACGTGGA TCTCCCCTAC AGGGGCCAAT CCTTATGATA 480 AGCTGAAAAT GCTCGCCTTC TACCCTTCGC CAAGCGCTAC 360 AGTTCCTCGG TAACCGCCGA GAGGCACGGG 180 CTGTCATAAG AATTTTCAAG GLUTAMIC ACID) TTGAACCCTA CTTCTACGCC CGAGCCGAAG GGACAAGATA GAGACCAGTT CGTTTATGAA CTTCGATCTC CTATCTGAAA 840 900 780 420 720 660 540 300

GAGCTTGGGA AGGAGTTCCT

ACCGGCGAGA ACCTTGAGAG

AGTCGCCCGC

CTCTGGGACG

TCTCCCGCTC

CAGCACTGGC

AACCTCGTTG

AGTGGTTCCT CCTCAGGAAG

AAAAGGAGCT GGCCAGAAGA

AAGATGCGAA GGTCACATAC

TCCGATGGAG GCCCAGCTTT CTCGCTTAAT CGGCCAGTCC

GCCTATGAGA GGAATGAGCT GGCCCCGAAC AAGCCCGATG

GTGATCCACG GGCAAGATAA ATGGAGTTCC ACCGACGGAT ATAACGATGA CGGCAGAGCT ATGAAGGAGG AGACAGGTTG GACCCGACGA TACATCGTGC GCCGTTGCCA AGGATAGTCA GAGACGCAGG GGCTTCTACA AGGGCGCGCT TACAGGCAGA AGCAGATAAC AAGAAGTTAC CGAGGGTTCT TCAAGTATAT GGTACTGCAA GGAGTGTGCA GAGAGCGTAA GGGCCATCAA GATCCTGGCA AACAGCTACT ACGGTTACTA AGAAGAAGAT ACTTCCCAGG ATTTTAGATC GTTTGAGTGC TTGGCTGAAG CCGAAGGGAA AGCACAAGTA TCAAGGGCTC AGAGGTTGGC CAACGCGCGG ACTIGAGATT GTGAGGCGTG AACGCGGCTT TTTTTGCCAC CCATCAAGGA GATAGAGGAA AAGTACGGCT TTAAGGTAAT TTCTGAGAGC TGGGAGGATA GGCGACAGGG CGATACCGTT GAAGGCCACG ATTGACCCGA CTTCGGTTAC CGACGCCGAG CGCGAGAGGA GTCAAAATAC GAGGGATTTA AAGGACTACA AGGCAACCGG CGAAAAGCTG AGCAAGTACG AGGTTCCGCC TGAAGCTTTG CTAAAGGACG GTGACGTCGA CTTCGTCACG CAACGCCAAA AATACCTGGA GCCGATGCTG ATTTATCCCG AGCCTGCTTG ATGCAAGGAA TATGACGTTG CCTGTACCCC TCAATCATCA CTATGTAAAA GAGCCCGAGA GAGGGTTGTG CGCAAGGAAG ACCTGCGCTA CCAGAAGACG CTTCCGGGCG TACTACATTG AAGAAGAAGT G AGAACCAGGT GCCCTGGAAC ACTGGAGCGA ATGCGGTGAT CGCTTGAGCT AAACCGTCAA CGGCCTGGGG TCGAGAGGAA GAGACCTCCT CCCCACAGGT TCACCCACAA 2325 GAAGGCCGTG GATAGCGAAA TCTCCCAGCC CGACGAGTTC GGTGATAAGC TCCCCACGIT GGAGAAGCTG AGACGAGGAA CGAGTACGAG AAAGAAGGCT CTACAGCGAC AAGGGAGTAC CGGCTATGCA GCTCCTCGAT AGAGGAGAGG CGGCCACCGC GGAGAACATA CGTCTCGCCG 1860 1620 1920 1800 1680 2100 2040 1980 2160 1740 1560 1500 1440 1380

// GCA ACC ATC AAG GGT GGC ATG AAG AAA GTA GTA AAG ACC CTG TGG TTC GAG GGC CGT GTG GGC AAG ATG ATC TCC TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC AAG CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG CAG AAA AAG TGA ACC TAC GAC ATC AAG GAG GAG TCC CIG GGC AAG

07d - KOD DNA POLYMERASE fusion protein (Fig. 17-LLL)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

GCA ACC GTA AAG ATC AAG AAA GTA TGG GGT GGC CAG ATG AAG ACC GGC CTG TTC GAG CGT AAG CGT AAG GGT GCG GTA AGC GAA AAG GIG TAC AAA GGC GAA GAA AAA GAG GTA CAG AAA AAG // GGC AAG ATG ATC TCC TTC GAC GCG ACC GAC CCG TAC AAG GAC GAG ATC TCC GAG AAG GGC CTG

CTCCTGAAGG ACGATTCTGC CATTGAGGAA GTCAAGAAGA TAACCGCCGA GAGGCACGGG 180 AAGGAAAACG GCGAGTTTAA GATTGAGTAC GACCGGACTT TTGAACCCCTA CTTCTACGCC 120 ACGGTTGTAA CGGTTAAGCG GGTTGAAAAG GTTCAGAAGA AGTTCCTCGG GAGACCAGTT 240 //ATGATCCTCG ACACTGACTA CATAACCGAG GATGGAAAGC CTGTCATAAG AATTTTCAAG 60

GATACGCTCA GTGTACCTAG CGGCAGAGCT GCCTATGAGA CTCTGGGACG GAGCTTGGGA ACCGACGGAT ATAACGATGA AGGGCGCGCT CAGAAGATAA AGAAGAAGAT GAAGGCCACG ACCGGCGAGA ACCTTGAGAG AGTCGCCCGC GCCGTCTTCG ATTCAGAGGA. AAGCGCTGTG GTTGACGTCG AGCTACGCCG GTTGAGAGAA GACCCGACGA GCCGTTGCCA GTGATCCACG AGGATAGTCA GAGACGCAGG GGCAAGATAA GGCTTCTACA ATGGAGTTCC TACAGGCAGA TTCTGCAAGG TATCCTGTGA AAAGACCCGG GACATTGAAA AGACAGGTTG GTTTGAGTGC TACATCGTGC AGCACAAGTA TCAAGGGCTC AGCAGATAAC AAGAAGTTAC CGAGGGTTCT AACGCGGCTT TTTTTGCCAC CCATCAAGGA GGTACTGCAA GGAGTGTGCA GAGAGCGTAA GGGCCATCAA GATCCTGGCA AACAGCTACT ACTICCCAGG ATTIATCCCG ACAGAGAAGG ATTTTAGATC ATGAAGGAGG GGAATGAGCT GGCCCCGAAC AAGCCCGATG TCTCCCGCTC CAGCACTGGC AGGAGTTCCT TCCGATGGAG GCCCAGCTTT GTCAGCCGAA GGAGAAGGTT TACGCTGAGG TGGGCGACAG AAAAGCTCGG AGGGATTAGT GCCAATGGAA CAGCAGTTAT TGACATCTAC AACTCTACTT AGAGGTTGGC CAACGCGCGG TCAAGTATAT TAAGACGGAC ACGTTCTCAT TCTCGACGGA ACGAGGAAGG CTCTCTACCA TTCTGAGAGC AACCTACAAC GAGGGATTTA AATACCTGGA GCCGATGCTG GATAGAGGAA AAGTACGGCT TIGGCIGAAG CGACGCCGAG TGGGAGGATA CGAAAAGCTG CTTCGTCACG CAACGCCAAA GATAAACCTG GAGGGAGATG ATAAAGCGCT GGCCAGGGTG TGAGGGCGAG TACTCATCCG CGCGAGAGGA TGAAGCTTTG ACTTGAGATT ATGCAAGGAA CCTGTACCCC CTATGTAAAA GITIGCCGIC AATAAACTTC CTTCGGTTAC CCGAAGGGAA TACTACATTG GGCGACAGGG GTCAAAATAC AAGGACTACA ATTGACCCGA AACCTCGTTG CCCACATACA CGCAAGGAAG AGCAAGTACG GTGAGGCGTG AAGAAGAAGT CTTCCGGGCG TATGACGTTG GAGCCCGAGA TACTCGATGG GCCCTCGGAA ATAACTTGGA GAGTTCGCCG GGCGACGAGG AGCCTGCTTG CTAAAGGACG TCAATCATCA GGCGACAACT GAGTACGACA CAGGACXXXC GAAGTGAAGG CT //TAG 2325 ACCIGCGCIA CCAGAAGACG AGAACCAGGT TCTCCCAGCC CGATACCGTT GCCCTGGAAC GGTGATAAGC AGGCAACCGG AGGTTCCGCC GTGACGTCGA ATGCGGTGAT AGACGAGGAA CGCTTGAGCT AAACCGTCAA TTAAGGTAAT CGGCCTGGGG ACGGTTACTA GAGACCTCCT TCACCCACAA GAGGGTTGTG AAAAGGAGCT AGTGGTTCCT CTCGCTTAAT AAGATGCGAA AAATAACCAC GACGGATACA GGGATGGAAG TCGACTTCGC TCCTCCGTGT CAGCGATAAG ACTGGAGCGA TCGAGAGGAA CCCCACAGGT CGCTTGAGGC AGAACGTGGA AGGGGCCAAT AGCTGAAAAT TACCCTTCGC CGACGAGTTC GAAGGCCGTG GATAGCGAAA TCCCCACGTT GGAGAAGCTG CGAGTACGAG AAAGAAGGCT GCTCCTCGAT CTACAGCGAC AAGGGAGTAC CGGCTATGCA AGAGGAGAGG CGGCCACCGC CGTCTCGCCG GGAGAACATA GGCCAGAAGA 1140 CCTCAGGAAG CGGCCAGTCC GGTCACATAC AGCCTGGGAA CGTTTATGAA CTTCGATCTC CGAGCCGAAG TCTCCCCTAC GCTCGCCTTC CAAGCGCTAC GGACAAGATA CTATCTGAAA TGTGAAGGAG CCTTATGATA 1860 1800 1740 1620 1560 1500 2160 2100 2040 1680 1440 1380 1200 1080 1020 720 660 600 1980 1920 1320 1260 960 840 780 900

SSO7d-Vent DNA POLYMERASE FUSION PROTEIN (Fig. 17-MIMM)

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FIG IT MMM (CONT)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

GCA ACC GTA AAG ATC AAG AAA GTA TGG GGT GGC AAG ACC CIG GGC GAG TIC AAG AAG CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG CGT CAG AAA AAG // GTG GGC AAG ATG ATC TCC TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC TTC ACC TAC GAC GAG GGC CIG AAG

GATCTTTTCC CCCAAGATTC AAACGGGCAG GTCGATGTTG AGTTATGCCG GATATTGAAA AGGGAACATC GAAGTCTGGA CTTCTCAAAG ATGACTCCGC TATTGAGGAG ATAAAGGCAA TAAAGGGCGA AAAGAGAACG ATGCTCGATT GCAATGAGGC GGATATAGGT GTATCCCCAG GAAAATATCA CGGCGCTTAA AGGGTGGCAT CAAAGTGTAT ACGTATGAGC TATGAAGCAG AAAGACCCCG CTCATAGACA AAAACTGTGA GAGTGCTCGA ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CTATAATCCG AATTTTTAAG GATGAAGAGG GAGTATGAGG AAGAAAGCCA TATGCGGACA AGACACTACA GGGTATCCTA TGGGAAACAG GAACAACTTA ACGCGAGGAA GGGAGTTTAA AATAGAACTT GGGACGTCTC AAAAGCTGGG ATGTGATAAT GCAGGATAAC AAGAAAGCAT CAGTTGTGCG AGAGGATGGG TGTCCAATGA ATGAAGAAGA CGTTTTATCA AGCTCATTTT GCTTTTACTT AGGAATTCCT CIGACGGCII AGGCAAGATG ATAGGCAAAG TCTGCAAGGA ATACCCTTGA TTTATTTGGA TCGGGAAGGA TTTTAGGAAA AGGGCTTGAT CAGCIGIGGI AAGATATAAA CGAGCATCCC AACAAGGGGC TTGGAAGTAG GAGAGGATTC AAACTACATA AACTCCAAAC GATAAGAGAA ATAGAGGAAA GTACTCGAAG GAATGTGCTG AAAAGAGGGC CCTGGGAGGA TGAACTTGCA CCGAACAAAC GAGATCAAGC ATTCTTCCCC GAAAAAACTA GCCCAGTACT AACCAAAAGC AAGGACGATA AACCTCCCAA CGTATACGCT AGTTCGGCTT GTCTTAGGAA GGGACAAAGA ACATCCCGAA AACTTACAAT GGGGACAATT TTGATTTGCC GGCCAGAGTA ATCACATGGA TCCCATGGAG TGACATTTAC TGCAGTGAAA GTCAGGAAAA AATTTTTGGG TTATGCCACA ATACCCGGGG GGCTATTAAA GAAGAAAATG CTTTCCGGGC TTTCCGCAGT TGATAGTTTT GCTGTGGAAA TCAAGGGTAG AAGAGAAATG ATAAAGCGTT TTGTTCAAGT TGAGGGAGAT TATGTAAAAG GAATTTGGAA GACCCTCATT TTCAGCCCTA TTTGTTACAA AAAAGCGCTA TTGCTTGCAA AAATCCACAA TTTATTCCCT TGTAAGAATT CTGTACCCTT ACCGGCAACC ATGGAAGCTG AAATTAGGAG GGAGACGAGG AGCTTAAGCT GAATATGACA TACCCTTTGC CAAGACXXXC CAGCTATGCG TAAGGAGAGA AAAAGCCTGA AGTTCGGCTT AAAGCGTTAC ACAGCTATTA CCATACTCGG ACGATGTTGC CAATAATAGT AGCCAGAAAA CAATGGAAGA CAGAGGAAAT AAAATATCGA AGGGCGAGAT TTCCAGGTCT TIGACCCGAT AGCTGGCAAA CTGATGAGGA TCGTGGAGTG GCTTGAGCTT CGAAAAGAAA GAGACATGGA TTGGAGTGAG TGCAGTCATA ACTCATTAAA TAAGGTTCTT CGGCTATATG GGACTTAATT TCCGATAGTA TACTCACAAC AGGTTTGTGG GTATCTTTTA GCTGATAGGT TGCTAGGGCA TGCCGCTATA TGAGGCAGTT AATCCACTTT GTATCTCATA TGTTAAAGAA AATAATGATT CCTTGCCTTT 420 GGGCAAAATA AAGGGAAGTT CGCATGGGGG AGAGTATAAA TTTGCCGTAT CAAGCGTTAT 1380 1260 1320 720 660 1680 1500 1440 1020 900 840 780 480 1200 1140 1080 960 600 300

ACAGAATACG ATCCTAGAAA ACACAAGTAC GATCCGGACT ACTACATAGA AAACCAAGTT ATAATAAGCT ATATCGTTCT CAAAGGGAGC GGAAAGATAA GCGATAGGGT AATTTTACTT CCTCATGTCG GAAAAGCTTG AAAGCTGTAG ATAGCTAAGG AGACTCAGGC AAAGGTTTTA GAGGCTATAC TTAAAGAGGG AAGTGTTGAA CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GGTAG 2325 TTGCCGGCAG TACTTAGGAT ACTCGAAGCG TTTGGATACA GAAAGGAGGA CGATAGCAAA AAGACTTGCC GCAAGAGGGA TAAAAGTGAA ACCGGGCACA TTATCCATGA GCAGATTACC AGGGATTTAA AGGACTACAA AGCCATTGGC AAGTTGTTAG AGATGTTGTA GAGAAAATAG CAAAATACAG GGTTCCACTT TTTAAGGTAT 2280 2100 2040 2220 2160

vent DNA POLYMERASE - Sso7d FUSION PROTEIN (Fig. 17-NNN)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

GICGAIGIIG AGTTATGCCG GATATTGAAA TATGAAGCAG GATCTTTTCC CCCAAGATTC AAAGACCCCG CTCATAGACA AGGGAACATC GAAGTCTGGA AAAACTGTGA ATGATACTGG ACACTGATTA CATAACAAAA GATGGCAAGC CTATAATCCG AATTTTTAAG GCAATGAGGC GGATATAGGT GTATCCCCAG GAAAATATCA CGGCGCTTAA AGGGTGGCAT CAAAGTGTAT ACGTATGAGC TGGGAAACAG AAACGGGCAG CTTCTCAAAG GAACAACTTA CCTGGGAGGA TATGTAAAAG ACGCGAGGAA TTTTAGGAAA AGAGGATGGG AAAAGCTGGG ATGTGATAAT AACTTACAAT GGGGACAATT TTGATTTGCC GTATCTCATA CGTTTTATCA AGGGCTTGAT CAGCTGTGGT TGACATTTAC GAATATGACA TACCCTTTGC AGCTCATTTT CGAGCATCCC CAAGACXXXC CAGCTATGCG GGGCAAAATA GAGTGCTCGA ATGACTCCGC TATTGAGGAG ATAAAGGCAA TAAAGGGCGA GAGACATGGA GGGAGTTTAA AATAGAACTT GACCCTCATT TTCAGCCCTA TATATATGCT GGGACGTCTC CAGTTGTGCG TGTCCAATGA ATGAAGAAGA TTTATTTGGA TCGGGAAGGA AAGAAAGCAT AAGATATAAA TCTGCAAGGA ATACCCTTGA GAAAAAACTA GCCCAGTACT TGCAGTGAAA GTCAGGAAAA AATTTTTGGG AAGGGAAGTT GAAGAAAATG CTTTCCGGGC AAAAGAGGGC TGTAAGAATT ACGATGTTGC TTTCCGCAGT CTGTACCCTT TGAACTTGCA CCGAACAAAC GAGATCAAGC ACCGGCAACC ATTCTTCCCC AACCAAAAGC AAATTAGGAG AAGGACGATA AACCTCCCAA TGATAGTITT GCTGTGGAAA TCAAGGGTAG AATCCACTTT AGTICGGCTT GICTIAGGAA GGGACAAAGA ACATCCCGAA AAGAGAAATG ATAAAGCGTT GGCCAGAGTA ATCACATGGA TGAGGGAGAT GAATTTGGAA AGGGCGAGAT AATAATGATT TCCCATGGAG GGAGACGAGG AGCTTAAGCT CCTTGCCTTT AAATCCACAA TTTATTCCCT ATGGAAGCTG TTGACCCGAT CCATACTCGG CAATAATAGT TACTCACAAC AGCCAGAAAA AGGTTTGTGG TCGTGGAGTG AGCTGGCAAA CAATGGAAGA CAGAGGAAAT TGCCGCTATA CGTATACGCT TTGTTCAAGT AAAATATCGA CTGATGAGGA CGAAAAGAAA AGAGTATAAA GCTGATAGGT GTATCTTTTA TGCTAGGGCA TGAGGCAGTT TGTTAAAGAA CAAGCGTTAT GGACTTAATT TCCGATAGTA TTTGCCGTAT 1320 1260 1140 1020 900 840 720 660 600 1200 1080 960 780 480 420 360 540 240

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GGGTATCCTA

AGGCAAGATG

GTACTCGAAG

GAATGTGCTG

GGCTATTAAA TTGCTTGCAA

ACAGCTATTA

CGGCTATATG

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CGCATGGGGG

AAAGCGTTAC

ATAGGCAAAG

ATAGCTAAGG GATGAAGAGG GAGTATGAGG ATAATAAGCT GAAAAGCTTG AAAGCTGTAG AAGAAAGCCA TATGCGGACA CAAAGCTCAA AACAAACCGG CTTAGATGCA TGGCTCAAGA GG 2325 // TTGCCGGCAG ACAGAATACG CCTCATGTCG AGACACTACA TAGAGATGAC TACTTAGGAT ACTCGAAGCG TTTGGATACA GAAAGGAGGA ATCCTAGAAA ACACAAGTAC ATATCGTTCT CGATAGCAAA AAGTTGTTAG AGACTCAGGC GCAGGATAAC GCTTTTACTT AGGAATTCCT CTGACGGCTT TTATCCATGA CAAAGGGAGC GCAGATTACC AGATGTTGTA AAGACTTGCC GAGAGGATTC GATAAGAGAA ATAGAGGAAA AGTTCGGCTT TAAGGTTCTT AAAGGTTTTA AACAAGGGGC AAACTACATA TTATGCCACA ATACCCGGGG AAAAGCCTGA GATCCGGACT ACTACATAGA GGAAAGATAA GCGATAGGGT GCAAGAGGGA TAAAAGTGAA AGGGATTTAA AGGACTACAA AGCCATTGGC GAGAAAATAG CAAAATACAG GAGGCTATAC TTGGAAGTAG TTTGTTACAA AAAAGCGCTA AACTCCAAAC TAAGGAGAGA TTCCAGGTCT TTAAAGAGGG AAACCAAGTT AATTTTACTT ACCGGGCACA GGTTCCACTT AAGTGTTGAA TTGGAGTGAG TGCAGTCATA GCTTGAGCTT ACTCATTAAA TTTAAGGTAT 2220 2160 2100 2040 1980 1920 1860 1800 1740 1680

j

// GCA ACC GGC AAG AAG AAA ATG CTG GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC GTA ACC GAG AAG CAG AAA AAG // GGC CGT GCT GCG GTA AGC GAA AAG GAC GCG TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TGA CCG TAC GAC ATC AAG GAG TCC GAG CIG GGC AAG

Deep Vent- Ssod7 DNA polymerase fusion protein (Fig. 17-000)

ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) GAGCTCGGTA GCAATCTTCG TACCACGTGA ATGCAGAGGC **AAGAGGGCCG** AAAGATCCCG GTCGAGGTAG AGCTATGCTG GACATAGAAA CTAATAGACA AGAGAGCATT GAGGTATGGA GGCTGTACTT TGAACACCCT CAGGACXXXC CCGCAATAAG GGATAAGATA AAGATAGTGA GAATTATAGA TGCCGAAAAG GTAAGGAAGA CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGC AAAGAAAACG GCGAGTTTAA GGTTGAGTAC V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, GGGAGTTCTT GACTGGAGAG GAAAGCCAAA TTAGGAGAAC AAAAGCTCGG ATGTTATAAT ATGAGGAAGA CCCTCTATCA AAGGCCTAAT TCCAATGGAA GGCGATGAAG AGCTCAAGTT GCTCGCATTT CCGCAGTTAT TGACATCTTT GAGTACGACA TTGGGGATAT TTTCCAGCGA CCCAATGGAG GCCCAGCTTT CAAGGTTAGT GATAAACCTC GACAGCGGTG GATAAAGCTA CCCCTGGGAA TACCTACAAC GGCGATTCTT TCGACCTTCC GAGGGAGATG ATAAAGCGGT TCCTCAAGGT GATAAGGGAG AGCCAAAGTC CGAAGGGGAG AGTTGCAAAG GGAGAAAGTT GACAGAAACT TATTCAATGG TACGCTCACG CCAACATACA CCCTCGAGGC GAGATAAAGG GAAGGATACA ATAACGTGGA GAGTTCGCGA AGGGGCCCAT TATAATGATA AGGATGCAAA GGGACGGTAG AGTTCCTGGG GAGGCCGATT CGT (ALL POSSIBLE CODONS FOR ARGININE) AAAAGATCGA TTAGACCTTA CATTTACGCT AGATAGCTGA TTCCGTTCGC GGCCTGGGAC TGAGCCAAAG GAAGAGGTAC CGGCCAGCCC GGTAACGTAC AGTTTATGAC CTTTGACCTC CTATCTAGTT TCTCCCGTAC 1020 960 900 840 780 720 660 600 540 480 420 360 300 240 180 120

CTGTGGGATG

TTTCTAGGTC

TTCAACTGGC

AACTTGGTGG

AGTGGTACCT

CCTCAGGAAC

ACTAAACAGA GCCGTTCTTA GTAAAGATAG GCCCTAGAGT GACACAGATG GCAAAAGCCC GATTACAGGC CCGGATACGC TTCGATCTCA GGGTACATAG CTAGTTATT AAAGAAACCC GAAGGGAAGA GAGGGCTTCT TATATAGAGT AGGCAAGAAA AAGTTCTGCA CTAAGGGAGA GCCTACGAGA CAGGTCTTAC GGCATGGCTT AACATCAAGA AGAAG GAATATTAGA GGCCTTTGGG GGAAGCATAA GTATGACGCT GGAATGAATT TGCTGAGGGG AGACGGGCCA CAAAAAGGTT AGCCGCTAGA ACGAGCAGAT CACGAGGCCC TTAAGGAGGT "NACTGAAAAG AAGCAAAAGT CCTAGAGGCT TAATCACTAG GGGGCTTGAA ACGTGAGAGG TCGTAGATTA GACTCTACGC TGAACAGGGA GCTACGCTGG TCGTAAGGAA GTTGGTACTG AACGGGCAAT AGGACTTCCC TAGATTTCAG TAAAAAGGAA GGCTCCAAAC GTTCTTCGTG TATAAACGCC CACAATTCCT GGAACTGGAG TAAGGAGTGC CAAAATCCTG GATGAAAGCT GGGGTTTATC AGGGTGTAGG GAGCCTGTAC GGGATACGTT AAGGAGCCGG TACAGGAAAG AAGACCTCAG GAGTATTACA ATAAGCAAGA GGAGTAAAGG ATCCTAAAGC ATAGTCAGGA ACGAAGAAGA AAGCCGGATG CTTCACGAGT CTGAGCAAGT AAGCTCCCAG GGGGCAAAAC GAAAAGTTCG GCAGAGAGCG GCAAACAGCT TCTAAAGACC CCCAGCCTGC GAATACGATG CCCTCGATAA TAGAAAATCA GGGCTATCCT TGAGGCCTGG ACAAGGCTAT ACGAAATACC ATGGCAACGT ATTATGGGTA GGGACTGGAG AGTATGCGTT TTACGGCCTG TCGCCCCAGA GGCTGTTGGA CCGAGGAGAT GGTTCAAAGT CAATCGAGAA TCAAGAGGTT TAATCACCCA AGAAAGGGCT AGAGGGAGTA GTGGCAGAAG GGTTTTACCT TGCAGAGGAG CATGGTGATA AGGTCCGCAC GATAGATGAG GCTTGAGTAC AAAGAAGAAA GGGGAGGGAA TCCAGAAAAG TGAGGAGGCA CGAAATAGCC CTTATACATA TTATGGGTAC GAAGATGCTT ATTGGATGAA GGTTGGGCAC TAACGTCTCA CGAGAGAAGO CTGGGAGGG 2280 2160 1920 1860 1800 1740 1620 2100 2040 1980 1680 1560 1500 1440 1380 1320 1260 1200

// GCA ACC GTA AAG ATC AAG AAA GGC AAG GTA ACC TTC GAG GGC TGG AAG CGT AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC CGT GTG GGC AAG ATG GGT GCG GTA AGC GAA CAG AAA AAG ATC AAG TCC GAC GCG CCG TTC ACC TAC GAC AAG TCC GAG GAG GGC CIG AAG

Ssod7 - Deep Vent DNA polymerase fusion protein (Fig. 17-PPP)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE)

/GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC AAG AAA GTA GGT GGC CAG AAG ACC GGC GAG TGG CGT CGT AAG GIG CAG GGT GCG GGC AAA AAG GTA AGC GAA AAG GAC GCG AAG ATG ATC TCC TTC ACC CCG TAC ATC GAC GAG TCC GAG GGC AAG CIG

AAAGAAAACG GCGAGTTTAA GGTTGAGTAC ATGATACTTG ACGCTGACTA CATCACCGAG GATGGGAAGC CGATTATAAG GATTTTCAAG CTCCTCAAAG ATGACTCGCA GATTGATGAG GTTAGGAAGA TAACCGCCGA GAGGCATGGG GACAGAAACT TTAGACCTTA CATTTACGCT 120

180

60

GCCGTTCTTA (ACTAAACAGA (GGGTACATAG :		CTAGTTATTT /	GTAAAGATAG	AAAGAAACCC I	GAAGGGAAGA :	GAGGGCTTCT /	GCCCTAGAGT :	GACACAGATG (TATATAGAGT :	GCAAAAGCCC (GATTACAGGC /	AGGCAAGAAA :	AAGTTCTGCA 1	CCGGATACGC :	TTAGTTTCCC :	CTAAGGGAGA (GCCTACGAGA (CTGTGGGATG :	GAGCTCGGTA (ACTGGAAAGG (GCAATCTTCG (TACCACGTGA 7	ATGCAGAGGC :	AAGAGGGCCG /	AAAGATCCCG !	GTCGAGGTAG 7	AGCTATGCTG 1	GACATAGAAA (CTAATAGACA !	AGAGAGCATT (GAGGTATGGA (AAGATAGTGA O
GAATATTAGA CAGGTCTTAC	GGAAGCATAA	TGCTGAGGGG	CAAAAAGGTT	ACGAGCAGAT	TTAAGGAGGT	AAGCAAAAGT	TAATCACTAG	ACGTGAGAGG	TCGTAGATTA	GACTCTACGC	TCGTAAGGAA	GTTGGTACTG	AACGGGCAAT	TAAAAAGGAA	AGGACTTCCC	TGAACAGGGA	TAGATTTCAG	GCTACGCTGG	GGAATGAATT	TTTCTAGGTC	GGGAGTTCTT	GACTGGAGAG	GAAAGCCAAA	TTAGGAGAAC	TTGGGGATAT	AAAAGCTCGG	ATGTTATAAT	TTTCCAGCGA	ATGAGGAAGA	CCCTCTATCA	AAGGCCTAAT	CCGCAGTTAT	GGCTGTACTT	GAATTATAGA
GGCCTTTGGG GGCATGGCTT	GTATGACGCT	AGACGGGCCA	AGCCGCTAGA	CACGAGGCCC	AACTGAAAAG	CCTAGAGGCT	GGGGCTTGAA	GTTCTTCGTG	TATAAACGCC	CACAATTCCT	GGAACTGGAG	TAAGGAGTGC	CAAAATCCTG	GATGAAAGCT	GGGGTTTATC	AGGGTGTAGG	GAGCCTGTAC	GGGATACGTT	GGCTCCAAAC	TTCAACTGGC	CCCAATGGAG	AGTTGCAAAG	GGAGAAAGTT	GATAAACCTC	GACAGCGGTG	GATAAAGCTA	TACCTACAAC	GAGGGAGATG	AGCCAAAGTC	CGAAGGGGAG	TCCAATGGAA	TGACATCTTT	TGAACACCCT	TGCCGAAAAG
TACAGGAAAG AACATCAAGA	GAGTATTACA	ATAAGCAAGA	GGAGTAAAGG	CTTCACGAGT	CTGAGCAAGT	ATCCTAAAGC	ATAGTCAGGA	ACGAAGAAGA	AAGCTCCCAG	GGGGCAAAAC	GAAAAGTTCG	GCAGAGAGCG	GCAAACAGCT	TCTAAAGACC	CCCAGCCTGC	GAATACGATG	CCCTCGATAA	AAGGAGCCGG	AAGCCGGATG	AACTTGGTGG	GCCCAGCTTT	TATTCAATGG	TACGCTCACG	CCAACATACA	GAGATAAAGG	CCCCTGGGAA	GGCGATTCTT	ATAAAGCGGT	ATAACGTGGA	GAGTTCGCGA	GGCGATGAAG	GAGTACGACA	CAGGACXXXC	GTAAGGAAGA
AAGACCTCAG AGAAG TAA	TAGAAAATCA	GGGCTATCCT	TGAGGCCTGG	ACAAGGCTAT	ACGAAATACC	ATGGCAACGT	GGGACTGGAG	AGTATGCGTT	GGCTGTTGGA	CCGAGGAGAT	GGTTCAAAGT	TTACGGCCTG	ATTATGGGTA	CAATCGAGAA	TCAAGAGGTT	TCGCCCCAGA	TAATCACCCA	AGAAAGGGCT	AGAGGGAGTA	AGTGGTACCT	CAAGGTTAGT	AGGATGCAAA	AGATAGCTGA	CCCTCGAGGC	GAAGGATACA	GGGACGGTAG	TCGACCTTCC	TCCTCAAGGT	AAAAGATCGA	AGGGGCCCAT	AGCTCAAGTT	TICCGTICGC	CCGCAATAAG	AGTTCCTGGG
GTGGCAGAAG	GGTTTTACCT	TGCAGAGGAG	CATGGTGATA	AGGTCCGCAC	TCCAGAAAAG	TGAGGAGGCA	CGAAATAGCC	GATAGATGAG	GCTTGAGTAC	AAAGAAGAAA	CTTATACATA	GGGGAGGGAA	TTATGGGTAC	GAAGATGCTT	ATTGGATGAA	GGTTGGGCAC	TAACGTCTCA	CTGGGAGGGG	CGAGAGAAGG	CCTCAGGAAG	CGGCCAGCCC	GGTAACGTAC	GGCCTGGGAG	AGTTTATGAG	CTTTGACCTC	TGAGCCAAAG	CTATCTAGTT	GATAAGGGAG	TCTCCCGTAC	TATAATGATA	GCTCGCATTT	GAAGAGGTAC	GGATAAGATA	GAGGCCGATT
2280 2328	22	2160	2100	2040	α	1920	O	0	4	α	1620	1560	1500	1440	1380	1320	1260	1200	1140	1080	1020	960	900	840	780	720	660	600	540	480	420	360	300	240

JDF-3 - Sso7d fusion protein (Fig. 17-QQQ)

V93E MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID) V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE

 $\mathtt{ACgcCGGTGGCTACGTCAAGGAGCCGGGGGGCCTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCTTCAATCATAATCACCCACAACGTCTCGCCAGATAC$ GTGGCTGAAGCCGAAGGAAGAAG// $\tt GTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCCGACGAAGCACAAGTACGATG$ $\tt GTACGAGGTTCCGCCGGAGAAGCTGGTTATCCACGAGCAGATAACGCGCGAGCTCAAGGACTACAAGGCCACCGGCCCGCACGTAGCCATAGCGAAgcGTTTGGCCGCCAGAGGT$ ${ t ACTGGAGCGAGATAGCGAAGGAAGGCGAGGGGTTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAA$ GAACTCGAATACGAGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAAGTACGCGGTCATCGACGAGGAGGGGCAAGATAACCACGCGCGGGCTTGAGATAGTCAGGCGCG $\tt CTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTC$ $\mathtt{CAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCCAACGAGCTACTACGCCTACTACGGCTACTACGGCT$ ${\tt GGTCGCGCCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCC}$ $\mathtt{ACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCCGCCGAGGGGCTTGAGAG$ ACCCTCGGGAGGGACGGGAGCCGAGGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAA ${\tt GCGCTTCTTGAGGGTCGTTAAGGAGGACCCCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTACCTGAAAAAAGCGCTGTGAGAAGCTTGGCGTGAGCTTT$ $\tt CAGGTCTGTGGAGGTCTGGGTCCTCTACTTCACGCACCCGCAGGACXXXCCGGCAATCCGCGACAAAATAAGGAAGCACCCCGCGGTCATCGACATCTACGAGTACGACATACCC$ ACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCCACGGCAGGGTCGTTAAGGGTTAAGCGCGCGGAGAAGGTGAAGAAAAGTTCCTC ${\tt CGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGAGGCAGGTCGGGCTTGGCGC$ ${ t TTCGCCAAGCGCTACCTCATAGACAAGGGCCTAATCCCGATGGAAGGTGAGGAAGAGCTTAAACTCATGTCCTTCGACATCGAGGACCCTCTACCACGAGGGAGAAGAGTTTGGAA$

//GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC AAG ATC AAG AAA GTA TGG CGT GTG GGC AAG ATG ATC TCC TTC ACC TAC GAC GAG GGC GGT GGC AAG ACC CIG GGC GAG AAG CAG AAA AAG CGT GGT GCG GTA AGC GAA AAG GAC GCG CCG AAG GAG CTG

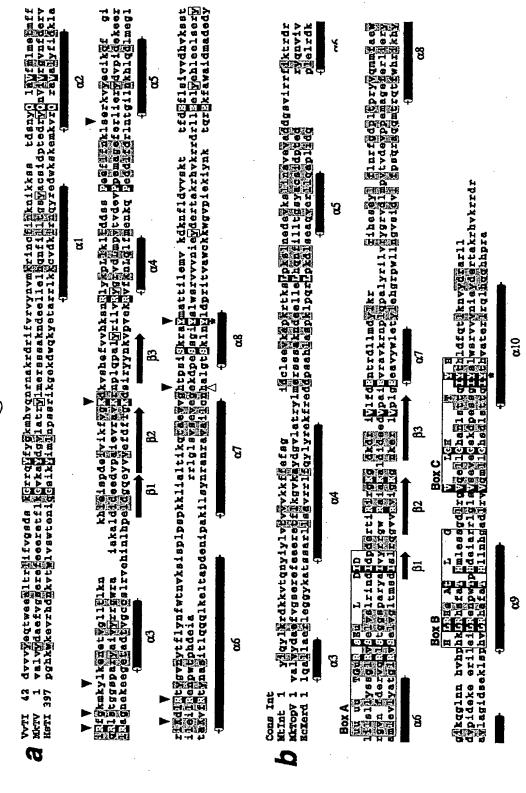
so7d - JDF-3 fusion protein (Fig. 17-RRR)

V93R MUTANT: XXX = AGA, AGG, CGA, CGC, CGG, CGT (ALL POSSIBLE CODONS FOR ARGININE) MUTANT: XXX = GAA, GAG (ALL CODONS FOR GLUTAMIC ACID)

//GCA ACC GTA AAG TTC AAG TAC AAA GGC GAA GAA AAA GAG GTA GAC ATC TCC AAG ATC AAG AAA GTA AAG ACC CIG GAG 999 TGG AAG CGT CGT GTG GGC AAG ATG ATC TCC GGT GCG GTA AGC GAA AAG CAG AAA AAG GAC TTC ACC GCG CCG TAC GAC GAG GGC AAG GAG CTG

rig. IT KRR (CONT)

 $\tt CGGACTACTACATCGAGAACCAGGTTCTGCCGGCAGTTGAGAGAATCCTCAGGGCCTTCGGCCTACCGCAAGGAAGACCTGCGCTACCAGAAGACGACGACGAGGTCGGGCTTGGCGC$ GTTAAAATCCGGCCCGGAACTGTGATAAGCTACATCGTTCTGAAGGGCTCCGGAAGGATAGGCGACAGGGCGATTCCCTTCGACGAGTTCGACCACGAAGCACAAGTACGATG $\mathtt{ACTGGAGCGAGATAGCGAAGGACGCAGGCGAGGGGTTTTGGAGGCGATACTCAGGCACGGTGACGTTGAAGAGGCCCGTCAGAATTGTCAGGGAAGTCACCGAAAAGCTGAGCAA$ GAACTCGAATACGAGGGCTTCTACGTCAGGGGCTTCTTCGTCACGAAGAAAAGTACGCGGTCATCGACGAGGAGGGGCAAGATAACCACGCGCGGGGCTTGAGATAGTCAGGCGCG $\tt CTATGCAGACAGACGGTCTCCATGCCACCATTCCTGGAGCGGACGCTGAAACAGTCAAGAAAAGGCAATGGAGTTCTTAAACTATATCAATCCCAAACTGCCCGGCCTTCTC$ $\textbf{ATGCCAGGGCAAGATGGTACTGCAGGGAGTGCGCCGAGAGCGTTACGGCATGGGGAAGGGAGTACATCGAAATGGTCATCAGAGAGCTTGAGGAAAAGTTCGGTTTTAAAGTCCT$ $\mathsf{CAGAAGATAAAGAGGAAGATGAAGGCAACTCTCGACCCGGCTGGAGAAGAATCTCCTCGATTACAGGCAACGCCCATCAAGATTCTCGCCAACAGCTACTACGGCTACTACGGCT$ ${\tt GCTCAACCGCGAGGGTGTAGGAGCTACGACGTTGCCCCCGAGGTCGCTCACAAGTTCTGCAAGGACTTCCCCGGCTTCATTCCGAGCCTGCTCGGAAACCTGCTGGAGGGAAAGG$ $\mathtt{ACgcCGGTGGCTACGTCAAGGAGCCGGAGCGGGACTGTGGGACAATATCGTGTATCTAGACTTTCGTAGTCTCTACCCTTCAATCATAATCACCCACAACGTCTCGCCAGATAC$ GGTCGCGCGCTACTCGATGGAGGACGCGAGGGTTACCTACGAGCTTGGCAGGGAGTTCTTCCCGATGGAGGCCCAGCTTTCCAGGCTCATCGGCCAAGGCCTCTGGGACGTTTCC $\mathtt{ACCTCCCGACCTACACCCTTGAGGCTGTATACGAGGCGGTTTTCGGCAAGCCCCAAGGAGAAGGTCTACGCCGAGGAGATAGCCACCGCCTGGGAGACCCGCCGAGGGCCTTGAGAG$ ${\tt ACGCGCTCCTCAGGGACGACTCTGCCATCGAAGAAATCAAAAAGATAACCGCGGAGAGGCACGGCAGGGTCGTTAAGGTTAAGCGCGCGGAGAAGAAGAAAAAGTTCCTCGG$ GTGGCTGAAGCCGAAGGGGAAGAAGAAGTGA ACCCTCGGGAGGGACGGGAGCCGAAGATACAGCGCATGGGGGACAGGTTTGCGGTCGAGGTGAAGGGCAGGGTACACTTCGACCTTTATCCAGTCATAAGGCGCACCATAA ${ t GCGCTTCTTGAGGGTCGTTAAGGAGAAGGACCCCGGACGTGCTGATAACATACAACGGCGACAACTTCGACTTCGCCTAACCTGAAAAAGCGCTGTGAGAAGCTTTGGCGTGAGCCTTT$ ${ t t T C G C C A A G C C T C T T A G A C A A G G G C C T A A T C C C G A T G G A G G G A G A G G A G G A G G A G G A G G A G G A G G A G G A G G A G G A G A G G A G G A G G A G G A G G A G G A G G A G G A G G A G G A G$ $\mathtt{ATGATCCTTGACGTTGATTACATCACCGAGAATGGAAAGCCCGTCATCAGGGTCTTCAAGAAGGAGAACGGCGAGTTCAGGATTGAATACGACCGCGAGTTCGAGCCCTACTTCT$



186

16.1

Figure 18 (cited from Belova et al. (2001) Proc. Natl. Acad. Sci. 98: 6015-6020)

Synthetic Sso7d gene

GCAACCGTAAAGTTCAAGTACAAAGGCGAAGAAAAAGAGĞTAGACATCTCCAA
GATCAAGAAAGTATGGCGTGTGGGCAAGATGATCTCCTTCACCTACGACGAGGG
CGGTGGCAAGACCGGCCGTGGTGCGGTAAGCGAAAAGGACGCGCCGAAGGAGC
TGCTGCAGATGCTGGAGAAG
CAGAAAAAG

The amino acid sequence of Sso7d.

ATVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDAPKELLQ MLEKQKK

The DNA sequence encoding the Sso7d-∆Taq fusion protein

ATGATTACGAATTCGAGCGCAACCGTAAAGTTCAAGTACAAAGGCGAAGAAAA 15 GAGGTAGACATCTCCAAGATCAAGAAAGTATGGCGTGTGGGCAAGATGATCTCC TTCACCTACGACGAGGGCGGTGGCAAGACCGGCCGTGGTGCGGTAAGCGAAAAG GACGCGCCGAAGGAGCTGCTGCAGATGCTGGAGAAGCAGAAAAAGGGCGGCGG TGTCACTAGTCCCAAGGCoCTGGAGGAGGCCCCCTGGCCCCGCCGGAAGGGGCC TTCGTGGGCTTTGTGCTTTCCCGCAAGGAGCCCATGTGGGCCGATCTTCTGGCCCT 20 GGCCGCCGCGGGGCCGGGCCCCCGAGCCTTATAAAGCCCT CAGGGACCTGAAGGAGGCGCGGGGGCTTCTCGCCAAAGACCTGAGCGTTCTGGC CCTGAGGGAAGGCCTTGGCCTCCCGCCCGGCGACGACCCCATGCTCCTCGCCTAC CTCCTGGACCCTTCCAACACCCCCCGAGGGGGTGGCCCGGCGCTACGGCGGG 25 GAGTGGACGGAGGGCGGGGGGGGGCCCCTTTCCGAGAGGCTCTTCGCC AACCTGTGGGGAGGCTTGAGGGGGGAGGAGGCTCCTTTGGCTTTACCGGGAG GTGGAGAGGCCCCTTTCCGCTGTCCTGGCCCACATGGAGGCCACGGGGGTGCGC CTGGACGTGGCCTATCTCAGGGCCTTGTCCCTGGAGGTGGCCGAGGAGATCGCCC GCCTCGAGGCCGAGGTCTTCCGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCG 30 GGACCAGCTGGAAAGGGTCCTCTTTGACGAGCTAGGGCTTCCCGCCATCGGCAA GACGGAGAAGACCGCCAAGCGCCCACCAGCGCCGCCGTCCTGGAGGCCCTCCG CGAGGCCCACCCATCGTGGAGAAGATCCTGCAGTACCGGGAGCTCACCAAGCT GAAGAGCACCTACATTGACCCCTTGCCGGACCTCATCCACCCCAGGACGGCCG CCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGGCAGGCTAAGTAGCTC

CGATCCCAACCTCCAGAACATCCCCGTCCGCACCCCGCTTGGGCAGAGGATCCGC CGGGCCTTCATCGCCGAGGAGGGGTGGCTATTGGTGGCCCTGGACTATAGCCAG ATAGAGCTCAGGGTGCTGGCCCACCTCTCCGGCGACGAGAACCTGATCCGGGTCT TCCAGGAGGGGGGGCACATCCACACGGAGACCGCCAGCTGGATGTTCGGCGTCC CCGGGAGGCGTGGACCCCTGATGCGCCGGGCGGCCAAGACCATCAACTTCG 5 GGGTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGCTAGCCATCCCTTA CGAGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTTCCCCAAGGTGCGG GCCTGGATTGAGAAGACCCTGGAGGAGGGCAGGAGGCGGGGGTACGTGGAGAC CCTCTTCGGCCGCCGCCGCTACGTGCCAGACCTAGAGGCCCGGGTGAAGAGCGT GCGGGAGGCGAGCGCATGGCCTTCAACATGCCCGTCCAGGGCACCGCCGC 10 CGACCTCATGAAGCTGGCTATGGTGAAGCTCTTCCCCAGGCTGGAGGAAATGGG GGCCAGGATGCTCCTTCAGGTCCACGACGAGCTGGTCCTCGAGGCCCCAAAAGA GAGGCGGAGGCCGTGGCCGGCTGGCCAAGGAGGTCATGGAGGGGGTGTATCC CCTGGCCGTGCCCTGGAGGTGGAGGTGGGGATAGGGGAGGACTGGCTCTCCGC CAAGGAGGCATTGATGGCCGCGGCGGAGGCGGCATCATCATCATCATTA 15 Α

The amino acid sequence of Sso7d-\Delta Taq fusion protein

MITNSSATVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDA

20 PKELLQMLEKQKKGGGVTSPKALEEAPWPPPEGAFVGFVLSRKEPMWADLLALAA
ARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLALREGLGLPPGDDPMLLAYLLDP
SNTTPEGVARRYGGEWTEEAGERAALSERLFANLWGRLEGEERLLWLYREVERPLS
AVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFRLAGHPFNLNSRDQLERVLF
DELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKSTYIDPLPDLIH
25 PRTGRLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFIAEEGWLLVALDYS
QIELRVLAHLSGDENLIRVFQEGRDIHTETASWMFGVPREAVDPLMRRAAKTINFGV
LYGMSAHRLSQELAIPYEEAQAFIERYFQSFPKVRAWIEKTLEEGRRRGYVETLFGRR
RYVPDLEARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLEEMGARMLL
QVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGIGEDWLSAKEGIDGR
30 GGGGHHHHHHH

The DNA sequence encoding the Sso7d-Taq fusion protein
ATGATTACGAATTCGAGCGCAACCGTAAAGTTCAAGTACAAAGGCGAAGAAAAA

GAGGTAGACATCTCCAAGATCAAGAAAGTATGGCGTGTGGGCAAGATGATCTCC TTCACCTACGACGAGGCGGTGGCAAGACCGGCCGTGGTGCGGTAAGCGAAAAG GACGCGCCGAAGGAGCTGCTGCAGATGCTGGAGAAGCAGAAAAAGGGCGGCGG TGTCACTAGTGGGATGCTGCCCCTCTTTGAGCCCAAGGGCCGGGTCCTCCTGGTG 5 GACGCCACCACCTGCCTACCGCACCTTCCACGCCCTGAAGGGCCTCACCACCA GCCGGGGGAGCCGGTGCAGGCGGTCTACGGCTTCGCCAAGAGCCTCCTCAAGG CCCTCAAGGACGGGGACGCGGTGATCGTGGTCTTTGACGCCAAGGCCCCCT CCTTCCGCCACGAGGCCTACGGGGGGTACAAGGCĞGGCCGGGCCCCACGCCAG AGGACTTTCCCCGGCAACTCGCCCTCATCAAGGAGCTGGTGGACCTCCTGGGGCT GGCGCGCCTCGAGGTCCCGGGCTACGAGGCGGACGACGTCCTGGCCAGCCTGGC 10 CAAGAAGGCGGAAAAGGAGGGCTACGAGGTCCGCATCCTCACCGCCGACAAAG ACCTTTACCAGCTCCTTTCCGACCGCATCCACGTCCTCCACCCCGAGGGGTACCT CATCACCCCGGCCTGGCTTTGGGAAAAGTACGGCCTGAGGCCCGACCAGTGGGC CGACTACCGGGCCCTGACCGGGGACGAGTCCGACAACCTTCCCGGGGTCAAGGG 15 CATCGGGGAGAAGACGCCGAGGAAGCTTCTGGAGGAGTGGGGGGAGCCTGGAAG CCCTCCTCAAGAACCTGGACCGGCTGAAGCCCGCCATCCGGGAGAAGATCCTGG CCCACATGGACGATCTGAAGCTCTCCTGGGACCTGGCCAAGGTGCGCACCGACCT GCCCCTGGAGGTGGACTTCGCCAAAAGGCGGGAGCCCGACCGGGAGAGGCTTAG GGCCTTCTGGAGAGGCTTGAGTTTGGCAGCCTCCTCCACGAGTTCGGCCTTCTG 20 GAAAGCCCCAAGGCcCTGGAGGAGGCCCCCTGGCCCCGCCGGAAGGGGCCTTC GTGGGCTTTGTGCTTTCCCGCAAGGAGCCCATGTGGGCCGATCTTCTGGCCCTGG CCGCCGCCAGGGGGCCCGGGCCCCCGAGCCTTATAAAGCCCTCA GGGACCTGAAGGAGGCGCGGGGGCTTCTCGCCAAAGACCTGAGCGTTCTGGCCC TGAGGGAAGGCCTTGGCCTCCCGCCCGGCGACGACCCCATGCTCCTCGCCTACCT CCTGGACCCTTCCAACACCACCCCGAGGGGGTGGCCCGGCGCTACGGCGGGA 25 GTGGACGGAGGGGGGGGGGGGCCGCCCTTTCCGAGAGGCTCTTCGCCAA CCTGTGGGGGAGGCTTGAGGGGGAGGAGGCTCCTTTGGCTTTACCGGGAGGT GGAGAGGCCCCTTTCCGCTGTCCTGGCCCACATGGAGGCCACGGGGGTGCGCCT GGACGTGGCCTATCTCAGGGCCTTGTCCCTGGAGGTGGCCGAGGAGATCGCCCG 30 CCTCGAGGCCGAGGTCTTCCGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCGG GACCAGCTGGAAAGGGTCCTCTTTGACGAGCTAGGGCTTCCCGCCATCGGCAAG ACGGAGAAGACCGCCAAGCGCCCACCAGCGCCGCCGTCCTGGAGGCCCTCCGC GAGGCCCACCCATCGTGGAGAAGATCCTGCAGTACCGGGAGCTCACCAAGCTG AAGAGCACCTACATTGACCCCTTGCCGGACCTCATCCACCCCAGGACGGGCCGCC

TCCACACCGGCTTCAACCAGACGGCCACGGCCACGGGCAGGCTAAGTAGCTCCG ATCCCAACCTCCAGAACATCCCCGTCCGCACCCCGCTTGGGCAGAGGATCCGCCG GGCCTTCATCGCCGAGGAGGGGTGGCTATTGGTGGCCCTGGACTATAGCCAGAT AGAGCTCAGGGTGCTGGCCCACCTCTCCGGCGACGAGAACCTGATCCGGGTCTTC CAGGAGGGGGGGACATCCACACGGAGACCGCCAGCTGGATGTTCGGCGTCCCC CGGGAGGCCGTGACCCCTGATGCGCCGGGCGCCAAGACCATCAACTTCGGG GTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGCTAGCCATCCCTTACG AGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTTCCCCAAGGTGCGGGC CTGGATTGAGAAGACCCTGGAGGAGGCAGGAGGCGGGGGTACGTGGAGACCC TCTTCGGCCGCCGCTACGTGCCAGACCTAGAGGCCCGGGTGAAGAGCGTGC 10 GGGAGCGCCGAGCGCATGCCTTCAACATGCCCGTCCAGGGCACCGCCGCCG ACCTCATGAAGCTGGCTATGGTGAAGCTCTTCCCCAGGCTGGAGGAAATGGGGG CCAGGATGCTCCTTCAGGTCCACGACGAGCTGGTCCTCGAGGCCCCAAAAGAGA GGGCGAGGCCGTGGCCGGCTGGCCAAGGAGGTCATGGAGGGGGTGTATCCCC TGGCCGTGCCCTGGAGGTGGAGGTGGGGATAGGGGAGGACTGGCTCTCCGCCA 15 AGGAGGCATTGATGGCCGCGGCGGAGGCGGCATCATCATCATCATTAA

The amino acid sequence of Sso7d-Taq fusion protein.

20

MITNSSATVKFKYKGEEKEVDISKIKKVWRVGKMISFTYDEGGGKTGRGAVSEKDA PKELLQMLEKQKKGGGVTSGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGE PVQAVYGFAKSLLKALKEDGDAVIVVFDAKAPSFRHEAYGGYKAGRAPTPEDFPRQ LALIKELVDLLGLARLEVPGYEADDVLASLAKKAEKEGYEVRILTADKDLYQLLSDR IHVLHPEGYLITPAWLWEKYGLRPDQWADYRALTGDESDNLPGVKGIGEKTARKLL EEWGSLEALLKNLDRLKPAIREKILAHMDDLKLSWDLAKVRTDLPLEVDFAKRREP 25 DRERLRAFLERLEFGSLLHEFGLLESPKALEEAPWPPPEGAFVGFVLSRKEPMWADL LALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLALREGLGLPPGDDPMLLA YLLDPSNTTPEGVARRYGGEWTEEAGERAALSERLFANLWGRLEGEERLLWLYREV ERPLSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFRLAGHPFNLNSRDOL ERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKSTYIDPL 30 PDLIHPRTGRLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFIAEEGWLLVA LDYSQIELRVLAHLSGDENLIRVFQEGRDIHTETASWMFGVPREAVDPLMRRAAKTI NFGVLYGMSAHRLSQELAIPYEEAQAFIERYFQSFPKVRAWIEKTLEEGRRRGYVETL FGRRRYVPDLEARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLEEMGA

RMLLQVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGIGEDWLSAKE GIDGRGGGGHHHHHH

The DNA sequence encoding the Pfu-Sso7d fusion protein

ATGATTTAGATGTGGATTACATAACTGAAGAAGGAAAACCTGTTATTAGGCTAT TCAAAAAGAGAACGGAAAATTTAAGATAGAGCATGATAGAACTTTTAGACCAT ACATTTACGCTCTTCTCAGGGATGATTCAAAGATTGAAGAAGTTAAGAAAATAAC GGGGGAAAGGCATGGAAAGATTGTGAGAATTGTTGATGTAGAGAAGGTTGAGAA AAAGTTTCTCGGCAAGCCTATTACCGTGTGGAAACTTTATTTGGAACATCCCCAA GATGTTCCCACTATTAGAGAAAAAGTTAGAGAACATCCAGCAGTTGTGGACATCT 10 TCGAATACGATATTCCATTTGCAAAGAGATACCTCATCGACAAAGGCCTAATACC AATGGAGGGGAAGAGACCTAAAGATTCTTGCCTTCGATATAGAAACCCTCTA TCACGAAGGAGAAGAGTTTGGAAAAGGCCCAATTATAATGATTAGTTATGCAGA TGAAAATGAAGCAAAGGTGATTACTTGGAAAAACATAGATCTTCCATACGTTGA GGTTGTATCAAGCGAGAGAGAGATGATAAAGAGATTTCTCAGGATTATCAGGGA 15 GAAGGATCCTGACATTATAGTTACTTATAATGGAGACTCATTCGACTTCCCATAT TTAGCGAAAAGGGCAGAAAAACTTGGGATTAAATTAACCATTGGAAGAGATGGA AGCGAGCCCAAGATGCAGAGAATAGGCGATATGACGGCTGTAGAAGTCAAGGG AAGAATACATTTCGACTTGTATCATGTAATAACAAGGACAATAAATCTCCCAACA 20 TACACACTAGAGGCTGTATATGAAGCAATTTTTGGAAAGCCAAAGGAGAAGGTA TACGCCGACGAGATAGCAAAAGCCTGGGAAAGTGGAGAGAACCTTGAGAGAGTT GCCAAATACTCGATGGAAGATGCAAAGGCAACTTATGAACTCGGGAAAGAATTC CTTCCAATGGAAATTCAGCTTTCAAGATTAGTTGGACAACCTTTATGGGATGTTT 25 AAAGAAACGAAGTAGCTCCAAACAAGCCAAGTGAAGAGGAGTATCAAAGAAGG CTCAGGGAGAGCTACACAGGTGGATTCGTTAAAGAGCCAGAAAAGGGGTTGTGG GAAAACATAGTATACCTAGATTTTAGAGCCCTATATCCCTCGATTATAATTACCC ACAATGTTTCTCCCGATACTCTAAATCTTGAGGGATGCAAGAACTATGATATCGC TCCTCAAGTAGGCCACAAGTTCTGCAAGGACATCCCTGGTTTTATACCAAGTCTC 30 TTGGGACATTTGTTAGAGGAAAGACAAAAGATTAAGACAAAAATGAAGGAAACT CAAGATCCTATAGAAAAAATACTCCTTGACTATAGACAAAAAGCGATAAAACTC TTAGCAAATTCTTTCTACGGATATTATGGCTATGCAAAAGCAAGATGGTACTGTA AGGAGTGTGCTGAGAGCGTTACTGCCTGGGGAAGAAGTACATCGAGTTAGTAT GGAAGGAGCTCGAAGAAAAGTTTGGATTTAAAGTCCTCTACATTGACACTGATG

GTCTCTATGCAACTATCCCAGGAGGAGAAAGTGAGGAAATAAAGAAAAAGGCTC TAGAATTTGTAAAATACATAAATTCAAAGCTCCCTGGACTGCTAGAGCTTGAATA TGAAGGGTTTTATAAGAGGGGATTCTTCGTTACGAAGAAGAGGTATGCAGTAAT AGATGAAGAAGGAAAAGTCATTACTCGTGGTTTAGAGATAGTTAGGAGAGATTG GAGTGAAATTGCAAAAGAAACTCAAGCTAGAGTTTTGGAGACAATACTAAAACA. CGGAGATGTTGAAGAAGCTGTGAGAATAGTAAAAGAAGTAATACAAAAGCTTGC CAATTATGAAATTCCACCAGAGAAGCTCGCAATATATGAGCAGATAACAAGACC ATTACATGAGTATAAGGCGATAGGTCCTCACGTAGCTGTTGCAAAGAAACTAGCT GCTAAAGGAGTTAAAATAAAGCCAGGAATGGTAATTGGATACATAGTACTTAGA 10 GGCGATGGTCCAATTAGCAATAGGGCAATTCTAGCTGAGGAATACGATCCCAAA AAGCACAAGTATGACGCAGAATATTACATTGAGAACCAGGTTCTTCCAGCGGTA CTTAGGATATTGGAGGGATTTGGATACAGAAAGGAAGACCTCAGATACCAAAAG ACAAGACAAGTCGGCCTAACTTCCTGGCTTAACATTAAAAAATCCGGTACCGGC GGTGCGGTGCAACCGTAAAGTTCAAGTACAAAGGCGAAGAAAAAGAGGTAGA CATCTCCAAGATCAAGAAGTATGGCGTGTGGGCAAGATGATCTCCTTCACCTAC 15 GACGAGGCGGTGCCAAGACCGCCGTGGTGCGGTAAGCGAAAAGGACGCGCC GAAGGAGCTGCTGCAGATGCTGGAGAAGCAGAAAAAGTGA

The amino acid sequence of the Pfu-Sso7d fusion protein

20 MILDVDYITEEGKPVIRLFKKENGKFKIEHDRTFRPYTYALLRDDSKIEEVKKITGERH GKIVRIVDVEKVEKKFLGKPITVWKLYLEHPODVPTIREKVREHPAVVDIFEYDIPFA KRYLIDKGLIPMEGEELKILAFDIETLYHEGEEFGKGPIIMISYADENEAKVITWKNID LPYVEVVSSEREMIKRFLRIIREKDPDIIVTYNGDSFDFPYLAKRAEKLGIKLTIGRDGS **EPKMQRIGDMTAVEVKGRIHFDLYHVITRTINLPTYTLEAVYEAIFGKPKEKVYADEI** 25 AKAWESGENLERVAKYSMEDAKATYELGKEFLPMEIQLSRLVGQPLWDVSRSSTGN LVEWFLLRKAYERNEVAPNKPSEEEYQRRLRESYTGGFVKEPEKGLWENIVYLDFR ALYPSIIITHNVSPDTLNLEGCKNYDIAPOVGHKFCKDIPGFIPSLLGHLLEERQKIKTK MKETODPIEKILLDYROKAIKLLANSFYGYYGYAKARWYCKECAESVTAWGRKYIE LVWKELEEKFGFKVLYIDTDGLYATIPGGESEEIKKKALEFVKYINSKLPGLLELEYE GFYKRGFFVTKKRYAVIDEEGKVITRGLEIVRRDWSEIAKETQARVLETILKHGDVEE 30 AVRIVKEVIQKLANYEIPPEKLAIYEQITRPLHEYKAIGPHVAVAKKLAAKGVKIKPG MVIGYIVLRGDGPISNRAILAEEYDPKKHKYDAEYYIENQVLPAVLRILEGFGYRKED LRYQKTRQVGLTSWLNIKKSGTGGGGATVKFKYKGEEKEVDISKIKKVWRVGKMIS FTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKK

The DNA sequence encoding the Sac7d-∆Taq fusion protein

ATGATTACGAATTCGACGGTGAAGGTAAAGTTCAAGTATAAGGGTGAAGAGAAA GAAGTAGACACTTCAAAGATAAAGAAGGTTTGGAGAGTAGGCAAAATGGTGTCC TTTACCTATGACGACAATGGTAAGACAGGTAGAGGAGCTGTAAGCGAGAAAGAT GCTCCAAAAGAATTATTAGACATGTTAGCAAGAGCAGAAAGAGAAGAAAGG CGGCGGTGTCACTAGTCCCAAGGCCCTGGAGGAGGCCCCCTGGCCCCGGCA AGGGCCTTCGTGGCTTTGTGCTTTCCCGCAAGGÄGCCCATGTGGGCCGATCTT CTGGCCCTGGCCGCCAGGGGGGGGCCGGGTCCACCGGGCCCCGAGCCTTAT AAAGCCCTCAGGGACCTGAAGGAGGCGCGGGGGCTTCTCGCCAAAGACCTGAGC 10 GTTCTGGCCCTGAGGGAAGGCCTTGGCCTCCCGCCGGCGACGACCCCATGCTCC TCGCCTACCTCCTGGACCCTTCCAACACCACCCCGAGGGGGTGGCCCGGCGCTA CGGCGGGGAGTGGACGGAGGGGGGGGGGGGGCCGCCCTTTCCGAGAGGC 15 CCGGGAGGTGGAGAGGCCCCTTTCCGCTGTCCTGGCCCACATGGAGGCCACGGG GGTGCGCCTGGACGTGGCCTATCTCAGGGCCTTGTCCCTGGAGGTGGCCGAGGA ACTCCCGGGACCAGCTGGAAAGGGTCCTCTTTGACGAGCTAGGGCTTCCCGCCAT CGGCAAGACGGAGAAGACCGGCAAGCGCTCCACCAGCGCCGCCGTCCTGGAGGC 20 CCTCCGCGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTACCGGGAGCTCAC CAAGCTGAAGAGCACCTACATTGACCCCTTGCCGGACCTCATCCACCCCAGGACG GGCCGCCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGCCAGGCTAAGT AGCTCCGATCCCAACCTCCAGAACATCCCCGTCCGCACCCCGCTTGGGCAGAGGA TCCGCCGGGCCTTCATCGCCGAGGAGGGGTGGCTATTGGTGGCCCTGGACTATAG 25 CCAGATAGAGCTCAGGGTGCTGGCCCACCTCTCCGGCGACGAGAACCTGATCCG GGTCTTCCAGGAGGGGGGGACATCCACACGGAGACCGCCAGCTGGATGTTCGG CGTCCCCGGGAGGCCGTGGACCCCCTGATGCGCCGGGCGGCCAAGACCATCAA CTTCGGGGTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGCTAGCCATC CCTTACGAGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTTCCCCAAGG 30 TGCGGGCCTGGATTGAGAAGACCCTGGAGGAGGCAGGAGGCGGGGGTACGTG GAGACCCTCTTCGGCCGCCGCCGCTACGTGCCAGACCTAGAGGCCCGGGTGAAG AGCGTGCGGAGGCGCGAGCGCATGGCCTTCAACATGCCCGTCCAGGGCACC GCCGCCGACCTCATGAAGCTGGCTATGGTGAAGCTCTTCCCCAGGCTGGAGGAA

ATGGGGGCCAGGATGCTCCTTCAGGTCCACGACGAGCTGGTCCTCGAGGCCCCA
AAAGAGAGGGCGGAGGCCGTGGCCCGGCTGGCCAAGGAGGTCATGGAGGGGT
GTATCCCCTGGCCGTGCCCCTGGAGGTGGAGGTGGGGATAGGGGAGGACTGGCT
CTCCGCCAAGGAGGGCATTGATGGCCGCGGGGGAGGCGGGCATCATCATCA
TCATTAA

The amino acid sequence of the Sac7d-\Delta Taq fusion protein

MITNSTVKVKFKYKGEEKEVDTSKIKKVWRVGKMVSFTYDDNGKTGRGAVSEKDA
PKELLDMLARAEREKKGGGVTSPKALEEAPWPPPEGAFVGFVLSRKEPMWADLLAL
AAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLALREGLGLPPGDDPMLLAYLL
DPSNTTPEGVARRYGGEWTEEAGERAALSERLFANLWGRLEGEERLLWLYREVERP
LSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFRLAGHPFNLNSRDQLERV
LFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKSTYIDPLPDLI
HPRTGRLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFIAEEGWLLVALDY
SQIELRVLAHLSGDENLIRVFQEGRDIHTETASWMFGVPREAVDPLMRRAAKTINFG
VLYGMSAHRLSQELAIPYEEAQAFIERYFQSFPKVRAWIEKTLEEGRRRGYVETLFGR
RRYVPDLEARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLEEMGARML
LQVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGIGEDWLSAKEGIDG
RGGGGHHHHHHH

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. The DNA sequence encoding the PL-ΔTaq fusion protein

GAGAGGCTCTTCGCCAACCTGTGGGGGAGGCTTGAGGGGGAGGAGAGGCTCCTT TGGCTTTACCGGGAGGTGGAGAGGCCCCTTTCCGCTGTCCTGGCCCACATGGAGG CCACGGGGGTGCGCCTGGACGTGGCCTATCTCAGGGCCTTGTCCCTGGAGGTGGC AACCTCAACTCCCGGGACCAGCTGGAAAGGGTCCTCTTTGACGAGCTAGGGCTTC CCGCCATCGCCAGGCGGGAGAGACCGGCAAGCGCTCCACCAGCGCCGCCGTCC TGGAGGCCTCCGCGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTACCGGG AGCTCACCAAGCTGAAGAGCACCTACATTGACCCCTTGCCGGACCTCATCCACCC CAGGACGGCCGCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGGCAG GCTAAGTAGCTCCGATCCCAACCTCCAGAACATCCCCGTCCGCACCCCGCTTGGG 10 CAGAGGATCCGCCGGGCCTTCATCGCCGAGGAGGGGTGGCTATTGGTGGCCCTG GACTATAGCCAGATAGAGCTCAGGGTGCTGGCCCACCTCTCCGGCGACGAGAAC CTGATCCGGGTCTTCCAGGAGGGGGGGGACATCCACACGGAGACCGCCAGCTGG ATGTTCGGCGTCCCCGGGAGGCCGTGGACCCCCTGATGCGCCGGGCGGCCAAG ACCATCAACTTCGGGGTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGC 15 TAGCCATCCTTACGAGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTT CCCCAAGGTGCGGGCCTGGATTGAGAAGACCCTGGAGGAGGCAGGAGGCGGG GGTACGTGGAGACCCTCTTCGGCCGCCGCCGCTACGTGCCAGACCTAGAGGCCC GGGTGAAGAGCGTGCGGGAGGCGCCGAGCGCATGGCCTTCAACATGCCCGTCC 20 AGGGCACCGCCGACCTCATGAAGCTGGCTATGGTGAAGCTCTTCCCCAGGCT GGAGGAAATGGGGCCAGGATGCTCCTTCAGGTCCACGACGAGCTGGTCCTCGA GGCCCAAAAGAGAGGGCGGAGGCCGTGGCCGGCTGGCCAAGGAGGTCATGG AGGGGGTGTATCCCCTGGCCGTGCCCCTGGAGGTGGAGGTGGGGATAGGGGAGG ACTGGCTCTCCGCCAAGGAGGCATTGATGGCCGCGGGGGAGGCGGGCATCATC 25 **ATCATCATCATTAA**

The amino acid sequence of PL-∆Taq fusion protein

MITNSKKKKKKKKKKKKKKKKKKKGGGVTSGATVKFKYKGEEKEVDISKIKKVWRVGK
MISFTYDEGGGKTGRGAVSEKDAPKELLQMLEKQKKGGGVTSPKALEEAPWPPPEG
AFVGFVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVL
ALREGLGLPPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEEAGERAALSERLFAN
LWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLE
AEVFRLAGHPFNLNSRDOLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIV

30

EKILQYRELTKLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLSSSDPNLQNIPVRTP LGQRIRRAFIAEEGWLLVALDYSQIELRVLAHLSGDENLIRVFQEGRDIHTETASWMF GVPREAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIPYEEAQAFIERYFQSFPKVR AWIEKTLEEGRRRGYVETLFGRRRYVPDLEARVKSVREAAERMAFNMPVQGTAAD LMKLAMVKLFPRLEEMGARMLLQVHDELVLEAPKERAEAVARLAKEVMEGVYPL AVPLEVEVGIGEDWLSAKEGIDGRGGGGHIHHHHH

PRIMER L71F

5'-CCTGCTCTGCCGCTTCACGC-3'

10

PRIMER L71R

5'-GCACAGCGGCTGGCTGAGGA-3'

PRIMER L18015F

15 5'-TGACGGAGGATAACGCCAGCAG-3'

PRIMER L23474R

5'-GAAAGACGA TGGGTCGCTAATACGC-3'

20

PRIMER L18015F

5'-TGACGGAGGATAAC GCCAGCAG-3'

. PRIMER L29930R

5'-GGGGTTGGAGGTCAATGGGTTC-3'

25

PRIMER L30350F

5'-CCTGCTCTGCCGCTTCACGC-3'

PRIMER L35121R

30 5'-CACATGGTACAGCAAGCCTGGC-3'

PRIMER L2089F

5'-CCCGTATCTGCTGGGA TACTGGC-3

PRIMER L7112R

5'-CAGCGGTGCTGACTGAATCATGG-3

PRIMER L30350F

5 5'-CCTGCCTGCCGCTTCACGC-3'

PRIMER L40547R

5'-CCAATACCCGTTTCA TCGCGGC-3'

10 PRIMER H-Amelo-Y

5'-CCACCTCATCCTGG GCACC-3'

. PRIMER H-Amelo-YR

5'-GCTTGAGGCCAACCATCAGAGC-3'

_ Human beta-globin primer 536F

5'-GGTTGGCCAATCTACTCCCAGG-3'

Human beta-globin primer 536R

20 5'-GCTCACTCAGTGTGGCAAAG-3'

Human beta-globin primer 1408R

5'-GATTAGCAAAAGGGCCTAGCTTGG-3'

25

15

PURIFIED THERMOSTABLE PYROCOCCUS FURIOSUS DNA POLYMERASE

AMINO ACID SEQUENCE (SEQ ID NO: 62)

Met Ile Leu Asp Val Asp Tyr Ile Thr Glu Glu Gly Lys Pro Val 10

Arg Leu Phe Lys Lys Glu Asn Gly Lys Phe Lys Ile Glu His Asp Arg 20 173/186

Thr Phe Arg Pro Tyr Ile Tyr Ala Leu Leu Arg Asp Asp Ser Lys 40 35

Glu Glu Val Lys Lys Ile Thr Gly Glu Arg His Gly Lys Ile Val Arg 50

FIG. 20A

Ile 80 Ile Val Asp Val Glu Lys Val Glu Lys Lys Phe Leu Gly Lys Pro 65

IlePro Thr 95 Gln Asp Val Tyr Leu Glu His Pro 90 Val Trp Lys Leu 85 Thr

Phe Glu Tyr 110 Arg Glu Lys Val Arg Glu His Pro Ala Val Val Asp Ile 100

174/186

Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro 115 $_{\rm m}$ Asp

Ile Glu Phe Asp 140 Glu Gly Glu Glu Leu Lys Ile Leu Ala 130 Met

160 Ile Met Pro Ile His Glu Gly Glu Glu Phe Gly Lys Gly 150 Tyr Leu 145

FIG. 20B

Ile	
Asn	175
Lys	
Trp	
\mathtt{Thr}	
Ile	
Val	170
Lys	
Ala	
Glu	
Asn	
	165
Asp	
Ala	
Tyr	
Ser	

Arg Glu Met Ile Lys	190
Val Val Ser Ser Glu A	185
Asp Leu Pro Tyr Val Glu V	180

Thr		
Val		
Ile		
Ile	205	
Asp		
Pro		
Asp		
Lys		
Glu	200	
Arg		
Ile		
Ile		
Arg		
Leu	195	
Phe		
Arg		
1-	15/1	8

Glu	
Ala	ŗ.
Arg	
Ş-	
Ala 1 220	
Leu Ala I 220	
e Pro Tyr	
Pro	
Phe	
Asp 215	
Phe	
Ser	
Asp	
\mathtt{Gly}	
Tyr Asn 210	
•	

FIG. 20C

Glu Pro Lys Phe Gly Lys 285 Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile 275

Ala Lys Ala Trp Glu Ser Gly Glu Asn 300 Tyr Ala Asp Glu Ile 295 Lys Val 290 176/186

Tyr320 Tyr Ser Met Glu Asp Ala Lys Ala Thr 315 Lys ' Leu Glu Arg Val Ala 305

Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg Leu 330

Ser Thr Gly Asn Leu 350 Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser 340

FIG. 20D

Ala	
Val Ala	
Glu	
Asn	365
Arg	
Glu	
Tyr Glu	
Ala	
Lys	360
Arg	
Leu	
Leu Leu	
Phe	
Trp	355
Glu	
Val	

g Leu Arg Glu Ser	
Arg	38
Arg	
Gln	
Tyr Gln	
Glu	
Glu	375
Glu	
Pro Ser	
Pro	
Lys	
Asn	370
Pro	

His Asn Val Ser Pro Asp Thr Leu Asn Leu Glu Gly Cys Lys Asn Tyr 420

Asp Ile Ala Pro Gln Val Gly His Lys Phe Cys Lys Asp Ile Pro Gly 445

FIG. 20E

460
455
•
450
7

Leu	80
	4
Leu	
Ile	
Lys	
Glu	
Ile	475
Pro	
Asp	
Gln	
\mathtt{Thr}	
Glu	470
Lys	
Met	
Lys	
Thr	
Lys	465

FIG. 20F

Lys 560	Leu
Lys Lys	Lys Leu Pro Gly Leu 575
Lys	$\mathtt{G1}\gamma$
ПЛе	Pro
Glu Glu Ile 555	Leu
Glu 555	Lys
Ser	Ser 570
Glu	Asn
Pro Gly Gly Glu Ser 550	Ile
$\mathtt{Gl}\mathtt{y}$	$\mathrm{Ty} r$
Pro 550	Lys Tyr
ПЛе	Val 565
\mathtt{Thr}	Phe
Ala	Glu
$\mathrm{Ty} r$	Leu Glı
Leu 545	Ala

Lys	Gly
Thr	Arq Gly
Val 590	Thr
Tyr Glu Gly Phe Tyr Lys Arg Gly Phe Phe Val 580	Ala Val Ile Asp Glu Glu Gly Lys Val Ile Thr
Phe	Val
G1y	Lys
Arg	G1y
m Lys 585	Glu
TYr	Glu
Phe	Asp
\mathtt{Gly}	Ile
Glu	Val
TYr 580	Ala
Glu	Tyr
Leu	Arg
Glu	Lys
179/1	86

605

009

595

Gln	
Thr	
Glu '	
Lys (
Ala I	620
Ile A	V
Glu 1	
Ser	
Trp 9	
Asp 7	515
Arg	_
l Arg	
Val	
Ile .	
Glu	610
Leu	

Ala Arg Val Leu Glu Thr Ile Leu Lys His Gly Asp Val Glu Glu Ala 625

FIG. 206

Ile	
G1u 655	
Tyr	
Asn	
Ala	
Leu Ala	
Lys 650	
Gln	
Ile	
Val	
Glu	
Lys 645	
val I	
Ile	
Arg	
Val	

Thr Arg Pro Leu His	670
Ile Tyr Glu Gln Ile	665
Pro Pro Glu Lys Leu Ala	099

la Lys Lys Leu Ala 685
ıl Ala
ı Val
Ala
Val
His 680
Y Pro
$\mathtt{Gl}_{\mathbf{y}}$
Ile
Ala
Lys 675
\mathtt{Tyr}
Glu
180/186

Tyr Ile Val		ţ.
Ile Gly	700	
Val		
Gly Met		
Pro		
e Lys	695	
ys Ile		
Val Lys		
\mathtt{Gly}		
a Lys	069	
Ala		

FIG. 20H

Ser Lys Glu Asp Leu Arg Tyr Gln Lys Thr Arg Gln Val Gly Leu Thr 755

Lys Ser Trp Leu Asn Ile Lys 770 181/186

Н PURIFIED THERMOSTABLE PYROCOCCUS FURIOSUS DNA POLYMERASE

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NUCLEOTIDE SEQUENCE (SEQ ID NO: 61)

120 ccotggtcct gggtccacat atatgttctt actcgccttt atgaagaatc ccccagtcgc tctaacctgg gttatagtga caaatcttcc tccaccaccg cccaagaagg ttatttctat

09

FIG. 20I

180	240	300	360	420	480	. 540	009	099	720	780
tatagagaag	tagatgtgga	acggaaaatt	gggatgattc	tgagaattgt	ggaaacttta	atccagcagt	acaaaggcct	aaaccctcta	cagatgaaaa	ttgtatcaag
taagtatagt	ataatgattt	aaaaaagaga	gctcttctca	ggaaagattg	attaccgtgt	gttagagaac	tacctcatcg	ttcgatatag	attagttatg	tacgttgagg
atgagatttt	tgtggggagc	taggctattc	atacatttac	ggaaaggcat	cggcaagcct	tagagaaaaa	tgcaaagaga	gattcttgcc	aattataatg	agatcttcca
tttctctctt	ttagtagata	aacctgttat	cttttagacc	aaataacggg	aaaagtttct	ttcccactat	atattccatt	aagagctaaa	gaaaaggccc	ggaaaaacat
cctcccctat	ccaaactgag	gaagaaggaa	catgatagaa	gaagttaaga	aaggttgaga	ccccaagatg	ttcgaatacg	gaggggaag	gaagagtttg	gtgattactt
caactctaca	gttttatact	ttacataact	taagatagag	aaagattgaa gaagttaaga	tgatgtagag	tttggaacat	tgtggacatc	aataccaatg	tcacgaagga	tgaagcaaag
				182	1186	,				

FIG. 205

FIG. 20K

1500

ctctaaatct

ttataattac ccacaatgtt tctcccgata

tagagcccta tatccctcga

FIG. 20L

FG. 20M

2880

ttgcaactct

gaaactttga cttctacaac atttctaact

agaaacatca

cagagaattg

3499					tgttcttgg	aagattgaga
3480	tcntcccnga	ttgttgtagc	aggaatgagg	atgcttcccc	cactactcag	gtagaagcgg
3420	gttgagaaaa	ccccatatgg	ctcactcaag	caatggtgtg	taccagacga	gacactcaaa
3360	cagactttta	agtgggtgtt	aagcttccaa	tccatatgat	ttggttgtgc	ctcgatttcc
3300	nngtcctctc	aggaggnnnn	tctttcatag	atgaaatttt	gtttttaagt	ccagggtaat
3240	tacttcatta	ttgacatttt	atgacaactc	gtctcaaatt	agaaataact	/ taacttttac
3180	tgtctctttt	aaaattgttt	tgacagatga	ttcttcttca	ttcaagctcc	A aaagtataat A aaagtataat A aaagtataat A aaagtataaat A aaaagtataat A aaaagtataataa A aaaagtataataa A aaaagtataataa A aaaagtaataataa A aaaagtaataataa A aaaagtaataataa A aaaagtaataataa A aaaagtaataataa A aaaagtaataataa A aaaagtaataataataa A aaaagtaataataataataataataataataataataata
3120	ttaactctgg	tttgaatcct	gtttgggatt	tctagccaag	tttgtattgc	ttccgggagt
3060	gatgctacct	aaggagagta	aagcaagcca	cctaatgtat	acaatttttt ccttgtatct	acaatttttt
3000	caagtccgct	ttcccgcacc	aacaccctg	tccaatggat	gcagagccgc	tttgctccaa
2940	gtagatcttt	tttcgacgac	tcctcgtcaa	tttaacggcc	tcaagatttt ctaaaagaat	tcaagatttt

FIS. 20N